The effect of dietary composition on the water balance of laying hens

Thesis

UNRESTRICTED

THE EFFECT OF DIETARY COMPOSITION ON THE WATER BALANCE OF LAYING HENS

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ABSTRACT

12 experiments measured quantitatively the response in excreta moisture of laying hens to dietary concentrations of nutrients and non nutrient components which met or exceeded recommended requirements. Experiments 1-4 measured the response in excreta moisture to dietary sodium, potassium, phosphorus and calcium. Experiment 5 examined the response to 2 sodium salts (bicarbonate or chloride) and for phosphorus x sodium interactions. Linear increases in excreta moisture were produced with increased dietary sodium (Y=719.65+8.12+-1.55X) potassium (Y=570.76+11.95+-2.01X) and phosphorus (Y=723.50+5.59+-0.308X). Dietary calcium concentration had no effect on excreta moisture. Experiments 6-9 measured the response in excreta moisture to dietary crude protein, lysine, methionine and amino acid availability. There were linear increases in excreta moisture with crude protein (Y=410.85+1.32+-0.076X) and lysine (Y=597.10+0.526+-0.073X). Methionine concentration had no effect on excreta moisture. Reduced amino acid availability gave a linear decrease in excreta moisture (Y=595.93+0.154+-0.048X). Experiments 10-11 measured the response in excreta moisture to dietary resistant starch, differing cereal sources and wheat bran concentration. Linear increases in excreta moisture (Y=679.97+0.170+-0.029X) were produced with increased dietary resistant starch. Cereal source had no effect but wheat bran increased excreta moisture. Experiment 12 measured the response in excreta moisture to dietary fat concentration, saturation and oxidation. There were no effects on excreta moisture. An experiment involving 1440 caged laying hens aimed to describe the change in dirty egg numbers with increases in excreta moisture. Raised excreta moisture gave linear increases in dirty egg numbers (Y=-19.92+0.0367+-0.00430X). A quantitative model was developed using experimental data to attempt prediction of dirty egg numbers from dietary nutrient composition. The nutrient composition of commercial feed samples were fitted and dirty egg numbers predicted. Predicted and commercially determined dirty egg numbers for each feed were incorporated in a regression analysis to validate the model. There was a significant linear relationship between predicted and commercially determined dirty egg numbers. Variation in dietary composition is therefore a major factor effecting excreta moisture and dirty egg output of caged laying hens.

DECLARATION

This thesis was composed by the author, and is a record of work carried out by him on an original line of research. All sources of information are shown in the texts and listed in the references; all help given by others is indicated in the acknowledgments.

None of this work has been presented in any previous application for a degree.

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ABSTRACT

High excreta moisture is a practical problem in egg production systems. In addition to affecting air quality, and increasing storage and disposal costs of poultry manure, high levels may increase faecal contamination of eggs. Dirty eggs are a continuing problem in the UK egg industry, eggs classified as dirty being downgraded. Any faecal contamination of the egg shell increases risk of contamination by bacterial organisms. Dietary composition is one factor known to effect excreta moisture. Considerable deviation in both dietary nutrient composition and concentration, from predicted dietary formulations, can occur through mixing errors and variation in the composition of raw ingredients used.

There is little data that quantitatively describes the variation in excreta moisture with differing concentrations of dietary nutrients, or their interactions in the laying hen. There is also little information that quantitatively describes the increase in the number of dirty eggs with raised moisture content of the excreta.

The first objective of this project was to quantitatively measure the excreta moisture of individually caged laying hens fed differing concentrations of dietary components, when these met or exceeded their recommended concentrations. A second objective was to quantitatively measure the effect of varying excreta moisture on the number of dirty eggs from caged laying hens and their microbial contamination. A final objective was to combine quantitative data in a mathematical model to predict differences in dirty egg numbers caused by diet.

Twelve experiments used either 48, 64 or 96 individually caged birds, arranged in four tiers, within an environmentally controlled room. Diets were fed for eight days. Water and feed intakes were measured and all excreta were collected on the final two days of each feeding period for

determination of excreta moisture.

Four experiments quantitatively measured the response in excreta moisture of laying hens to dietary concentrations of sodium, potassium, phosphorus and calcium. A fifth experiment examined whether excreta moisture differed when two different sodium salts (bicarbonate or chloride) were fed, and if the effects of dietary sodium and phosphorus excess were additive. Linear increases in the moisture content of excreta were produced with increased dietary concentrations of sodium (Y=719.65+8.12±1.55X), potassium (Y=570.76+11.95±2.016X) and phosphorus (Y=723.50+5.59±0.308X). Variation in dietary calcium had no effect on excreta moisture. Sodium and phosphorus acted additively, and the response to dietary sodium concentration was not altered by two different anions.

A second series of four experiments quantitatively measured the response in excreta moisture to dietary concentrations of:

(i) A well balanced source of crude protein

(ii) Lysine at constant crude protein concentration

(iii) Methionine at constant crude protein concentration

(iv) Amino acid availability

All were examined independently of dietary electrolyte concentration. Linear increases in the moisture content of excreta were produced with increased concentrations of crude protein (Y=410.85+1.32±0.076X) and lysine (Y=597.10+0.526±0.073X). Variation in methionine concentration had no effect on excreta moisture. Reduced amino acid availability gave a linear decrease in excreta moisture (Y=595.93+0.154±0.048X). Protein and lysine concentrations acted additively and there were no methionine x lysine concentration interactions.

Two experiments quantitatively measured the response in excreta moisture to dietary concentrations of different carbohydrate fractions. The first examined the effect of dietary concentrations of resistant starch. The second examined the effect of:

(i) Non - starch polysaccharide concentration

(ii) Soluble x insoluble non - starch polysaccharide interactions

This was achieved by comparing four cereal types and two concentrations of dietary wheat bran. Linear increases in excreta moisture were produced with increased dietary resistant starch as a proportion of total starch ($Y=679.97+0.170\pm0.029X$). Variation in cereal source had no effect on excreta moisture, but increased wheat bran concentration increased excreta moisture.

A final experiment, with individually caged laying hens quantitatively measured the response in excreta moisture to:

(i) Dietary concentrations of fat

(ii) Level of saturation of dietary fat

(iii) Exposure of dietary fat to oxidising condition

There were no effects of the concentration, level of saturation, or exposure to oxidising conditions of dietary fat, on excreta moistures.

An experiment involving 1440 caged laying hens was performed, that changed the excreta moisture by feeding diets with different levels of sodium. The objective of the experiment was to quantitatively describe the change in the number of dirty (excreta contaminated) eggs with increases in excreta moisture. Increased dietary concentrations of sodium gave linear increases in

the moisture contents of excreta. Raised excreta moisture gave linear increases in both the proportion of dirty eggs, ($Y = -19.92 + 0.0367 \pm 0.00430X$) and the microbial contamination of first quality egg shells ($Y = -2.36 + 0.0081 \pm 0.00094X$).

A model was developed using quantitative data from experimental work to attempt prediction of variation in dirty egg numbers from variation in dietary nutrient composition. Nutrient compositions of commercial feed samples were fitted to the model. To validate the model predicted dirty egg numbers were incorporated as independent variables in a regression analysis with commercially determined dirty egg numbers for each feed as dependent variables. There was a significant linear relationship ($r^2 = 0.22$) between dirty egg numbers predicted by variation in diet and determined commercially. It was concluded that variation in dietary composition is a major factor effecting excreta moisture and dirty egg output of caged laying hens.

CHAPTER 1.

INTRODUCTION

Water constitutes the greatest percentage of animal tissues, representing approximately 70 % of total body weight. Under normal physiological conditions, the water balance of the bird is maintained precisely, by balancing water gains against water loss (Hill *et al,* 1979). Any factor which influences water intake, or loss, will influence the water balance. The most common consequence of a disturbance in water balance in poultry is raised excreta moisture, variations in which occur either through increased urinary and or faecal elimination of water, both being voided through the cloaca.

High levels of excreta moisture may cause economic loss in egg production systems, although it may not affect the number of eggs, or the efficiency of egg production. High excreta moisture may increase the number of dirty eggs (eggs contaminated with excreta) both in caged systems with correct floor slopes and mesh specification (Appleby and Smith, 1991) and non caged systems. Under EC marketing regulations class A eggs should have a clean shell, and not have been cleaned, those soiled or washed being downgraded (Anon, 1993 a). A recent survey by the UK egg inspectorate of 2400 batches of eggs (Anon, 1993 b) indicated that 14 % of all batches had been washed and that 75 % of the washed eggs were from caged systems.

Contamination of the egg shell with excreta may also increase the risk of contamination by bacterial organisms (Humphrey *et al,* 1989). Various workers have shown a relationship between dirty eggs and increased microbial contamination (Forsyth *et al,* 1953; Johns and Berard, 1946). There are potential dangers to consumer health of using faecally contaminated eggs (Anon, 1988). The bacterial content of the shell may also be linked to the environment in which the egg is laid (Harry, 1963), and therefore there is the possibility of contamination of first quality eggs when exposed to raised excreta moisture.

High excreta moisture can also increase the relative humidity (Richards, 1976) and ammonia concentrations (Williams, 1995) in controlled environment houses, and provide a more favourable environment for fly larvae development. High relative humidity can lead to rapid deterioration of house structure and electrical equipment and increased survival of viruses which cause respiratory disease (Gloster, 1983). The increased volume of high moisture excreta produced could increase the storage and disposal costs of poultry manure from an egg production unit.

Dietary composition is a major factor that affects water balance and therefore the moisture content of excreta produced by laying hens. Dietary components that may affect excreta moisture include sodium (Hijikuro, 1976) potassium (Kando and Ross, 1962 a and b) calcium (Roland and Caldwell, 1985) magnesium (Stilmak and Sunde, 1971) resistant starch (Cooke and Raine, 1986), non - starch polysaccharides (Choct and Annison, 1992 a and b) protein (Patrick, 1955) and fat (Bray, 1985). However others have indicated that excreta moisture content is affected by behaviour associated with boredom (Hungerford, 1969; Lintern - Moore, 1972) mineral composition of water supply (Selye, 1943; Krista *et al,* 1961) environmental factors (Parker *et al,* 1972) physiological status (Van Kampen, 1974) housing type (Yoselswitz, 1991) and disease (Jordan, 1990).

Considerable deviation in dietary nutrient composition from predicted dietary formulations can occur. Variation occurs through mixing errors, variation in the type of raw ingredients used within diets, and deviation in nutrient composition of raw ingredients from values used in formulation packages through differences in origin, growing conditions, degree of processing and method of storage. Additionally some non nutrient components of diets are not formulated for and therefore are subject to considerable between diet variation. Variation in dietary components which

predispose layers to increased excreta moisture may be a possible cause of variability in dirty egg output.

There is little information that quantitatively describes the variation in excreta moisture with differing concentrations of dietary nutrients or their interactions in the laying hen and where work exists it tends to be ambiguous. There is also little information that quantitatively describes the increase in the number of dirty eggs with raised moisture content of the excreta.

The general objective of this project was to determine quantitatively the effect of variation in dietary nutrient composition on variation in moisture content of excreta, and the proportion of dirty eggs produced by caged laying hens, and to subsequently develop a quantitative model to enable prediction of the proportion of dirty eggs from dietary nutrient composition. Although directly applicable to caged laying hens the work will also have relevance to none caged egg flocks, breeder flocks producing hatching eggs and wet litter problems in broiler flocks.

The specific objectives of this project were:

(i) To quantitatively measure the response in water intake and excreta moisture of individually caged laying hens to dietary concentrations of nutrients, and non - nutrient components, when meeting or exceeding the recommended requirement, and to examine interactions between the various components.

(ii) To quantitatively measure the effect of varying excreta moisture on output of dirty eggs, and the degree of microbial contamination of first quality eggs in a caged laying flock.

(iii) To develop a quantitative model to predict the effect of variation in dietary composition on variation in excreta moisture and the proportion of dirty eggs produced by caged laying flocks, and to subsequently test its relevance in a commercial situation.

CHAPTER 2.

WATER BALANCE AND METABOLISM IN POULTRY

2.1. INTRODUCTION

Water constitutes the greatest percentage of plant and animal tissues, representing approximately 70 % of total body weight, although, due to age and fat deposition, it may vary between 50 and 80 % (Geogievskii *et al*, 1990). Of this body weight 70 % is intracellular, and 30 % extracellular, whilst 75 % of the latter is present in the interstitial space, and the remaining 25 % is found in the plasma (Houpt, 1970). The distribution of water in organs and tissues is not uniform. Approximately 55 % of all water is contained in muscle, 10 % in hide, 6-7 % in skeleton and blood, 5 % in the liver, and the remainder in soft tissue. Water fulfils a range of physiological and chemical functions and forms the basis of the transport systems that provide nutrients to the cells (Bailey, 1990). As a solvent, water is the body's major transportation medium for nutrients, metabolites, waste products, hormones and other chemical messengers. Having a high specific heat capacity, and heat conductivity, water plays a role in thermoregulation and homeothermy, and allows survival at high environmental temperatures. Water is a lubricant, and a component of reactions such as oxidation, hydrolysis, and acid base balance. Water is required for excretion of waste nitrogen, as birds excrete nitrogen as uric acid. Despite this importance, knowledge of animal water requirements are surprisingly limited. This is probably because water is abundant, inexpensive, and not traded commercially. This chapter will consider the water requirements of poultry, normal water balance, and the physiological factors involved in maintaining water balance.

2.2. WATER ABSORPTION

Hill (1977) states that whereas the major absorption site of food components is in the small intestine, the crop, colon and cloaca are also important in water and electrolyte absorption. Water

in mammals is generally suggested to be absorbed through a passive mechanism, dependant on osmotic pressure. However, in birds, recorded osmotic pressures in different regions of the gastrointestinal tract are considerably higher than in mammals, where different regions are isotonic, and may be more than twice that of the blood suggesting the existence of a specific mechanism or an active intestinal process in birds.

2.3. WATER BALANCE OF THE LAYING HEN

The water balance is the difference between water loss and water gain. Lintern-Moore (1972), Howard (1975) Osbaldiston (1969) and Dixon (1958) have used the term water balance, but considered only water intake and water loss in the urine and faeces. Hill *et al* (1979) suggested that complete water balance was more adequately explained by the following equation

EQUATION 2.3.1.

$$W_D + W_F + W_M - (W_E + W_d + W_e + W_u + W_f) = 0$$

W_D is water drunk, W_F water content of feed, W_M metabolic water, W_E water lost in eggs, W_d the water component of a change in body mass, W_e the water lost through evaporation, W_u the water in the urine and W_f water lost in faeces. Any factor which influences the value of any of the individual terms will affect the complete water balance. Hill (1977) attempted using her and other data to quantify each term in the equation. The resulting water balance is shown in table 2.1.. During experimentation, average house temperature was 18.6 °C and relative humidity 55 %. There are a number of factors contributing to the error (Hill *et al*, 1979). Firstly, the error associated with the estimation of individual terms, secondly, error arising from combining data

Table 2.1. Water balance of the laying hen according to the formula of Hill (1977) and adapted from Hill et al (1979)

Term	Value (g/bird/d)	Reference
W_D	+ 190	Hill (1977)
W_F	+ 13	
W_M[2]	+ 41	
W_E	- 34	
W_d	- 1.2	
W_e[3]	- 19	Van Kampen (1974)
	- 27	Barott and Pringle (1941)
	- 48	Richards (1976)
$W_u + W_f$	- 125	Dixon (1958)
		Medway and Kare (1959)

1. The data relate to Shaver 288 with the following characteristics: age, 22-42 weeks; weight, 1.6 kg; change in weight, + 1.9 g/d; food intake, 116 g/d; rate of lay, 0.85 eggs/d; weight of egg, 58.3 g; water content of egg, 683 g/kg.
2. Potential metabolic water available assuming the complete oxidation of relevant dietary constituents.
3. The relative humidity varied in each of the these experiments.

from several sources. For the stated set of conditions errors were likely to be the greatest in estimation of W_e, because none of the papers from which data was used stated experimental conditions and evaporative water loss is affected by various variables, such as temperature, humidity, air movement, animal movement, and acclimatization. Water lost by excretion was also estimated in conditions which differed from those of Hill (1977) although there was good agreement between the cited authors.

2.4. WATER INTAKE

The bird obtains water from three sources:

2.4.1. Water drunk Approximatly 70% of water is obtained through this route (Leeson and Summers, 1992). Table 2.2. lists a summary of published figures for water consumption. Considerable divergence is seen, as numerous factors interact with water intake. Consequently there is no single water requirement for the species.

2.4.2. Dietary water Most diets contain approximately 890 g/kg DM by weight, and therefore approximately 110 g/kg moisture. A typical laying hen consuming 120 g of feed per day, would therefore be expected to obtain around 13 g of water per day from this source. Bound water may also become available during digestion and metabolism, such that 7-8 % of total requirements could originate from the feed (Bailey, 1990). However Medway and Kare (1959) suggested that only 2.5 % of total water intake was obtained from food in layers.

2.4.3. Metabolic water Metabolic water is created as a by - product of catabolism of protein, carbohydrate and fat. Different amounts of water are produced from the oxidation of the various

Table 2.2. Previously published values for daily water intake of laying hens

Reference	Stock	Age	Mean water intake (g/bird/d)
Kendler and Taylor (1966)	Rhode Island Red	2-2.5 years	257
Dixon (1958)	White Leghorn	1 year	334
Favret *et al* (1967)	Unspecified		300
Raffel *et al* (1976)	White Leghorn	1 year	132
Lintern - Moore (1972)	Brown Leghorn Medium weight hybrids		116±28, 125±22, 121±19
Sturkie and Joiner (1959)	Unspecified		303
Osbaldiston (1969)	Light Sussex		426 (by extrapolation)
Heywang (1941)	White Leghorn	1st year of lay	319
Medway and Kare (1959)	White Leghorn		483±130

1 Means and standard errors are for three successive recording periods of one week duration

components. The contribution of this water source to the total intake will depend on the percentage of each dietary component fully oxidized by the bird (Hill *et al,* 1979). The potential contribution of each can be estimated from the dietary composition if the number of water equivalents of the dietary components are known. Such data was provided by Bartholomew (1963). If fats were metabolized (fully oxidized), then about 1.18 g of water were produced per gram of fat whereas protein and carbohydrate yielded about 0.60 and 0.50 g/g respectively. Favret *et al* (1967) estimated the metabolic water produced by hens to be about 60 g/bird/d, assuming an intake of 150 g food/b/day. Total metabolic water can, however, be more easily estimated from energy intake, since approximately 0.135 g of water are produced per kilocalorie of energy consumed (Kerstens, 1964). Thus, for a bird consuming 300 kcals ME per day, approximately 40 g of metabolic body water is produced, and enters the body pool. This represents about 15 % of the birds total water intake each day (Leeson and Summers, 1992).

2.5. PHYSIOLOGICAL CONTROL OF WATER INTAKE

As previously mentioned, control of water intake in the fowl is achieved by balancing water gain and water loss, in order to maintain the body water pool at a set point. Various models have been put forward to explain control (Mc Farland, 1965; Oatley, 1967). The majority explain water intake as a negative feedback mechanism, through which deviations in the body water pool from the set point are corrected. Mc Farland (1970) however, suggested that feed forward processes and positive feedback also exist.

2.5.1. Negative feedback As pointed out by Bligh (1976) set point regulatory systems require four components, a sensor, a co-ordinator, an effector, and a mechanism of feed back to the sensor. The sensor for water intake lies in the preoptic region (*lamina terminalis*) of the hypothalamus,

and is thought to be an osmoreceptor. Unlike in rat, where reductions in plasma volume, and increases in plasma osmolarity, both stimulate thirst and induce drinking, (Oatley, 1967; Fitzsimmons, 1961; 1969), in the fowl the response is mediated by extracellular hyper osmolarity only (Stallone and Braun, 1986). The hypothalamus itself acts as co-ordinator, by initiating drinking, and stimulating release of argenine vasotocin (avian antidiuretic hormone) from the neurohypophysis, which promotes reabsorption of water in the distal tubules and collecting ducts of the kidney nephrons, producing hyper osmotic urine. The kidneys also respond to a reduction in plasma volume by releasing the hormone rennin, which combines with plasma protein to form angiotensin I. This breaks down to form angiotensin II, a powerful vasoconstrictor which induces drinking (Bailey, 1990). Other hormones, including saralsin (an inhibitor of angiotensin II) and endogenous opiates have an inhibitory effect on water intake, whilst naloxone stimulates intake. Luteinising hormone also plays a role within the hypothalamus in drinking, its stimulation promoting hyperdipsia. These mechanisms act together to restore normal plasma osmolarity which reduces the stimulation of osmoreceptors.

2.5.2. Feedforward mechanisms These occur in anticipation of a deviation from the set point value. Hill *et al* (1979) suggested as an example, drinking in association with eating, rather than after eating. The absorption of the products of digestion increase osmolarity, and thereby initiate drinking. However a close temporal association between eating and drinking exists in the hen, and Oatley and Toates (1969), and Fitzsimmons and Le Magnen (1969), have pointed out that the time course of fluid movement is such that drinking associated with eating must occur in anticipation of the change in body fluids rather than as a response to them.

2.5.3. Positive Feedback Positive feedback promotes maintenance of drinking behavior once initiated. Mc Farland and Wright (1969) have shown that positive feedback is provided by oral

factors supplying reinforcement of drinking.

2.5.4. Satiation This depends on the total amount of water ingested and occurs whether or not the water is provided orally. However, water is only rewarding in an operant situation, when delivered through the mouth. Rolls (1975) suggested water receptors in the mouth mediate the activities of water reward neurons in the hypothalamus whenever a body water deficit exists.

2.6. WATER LOSS

Water losses occur via evaporative, urinary and faecal routes, and through the water component of new tissue growth. In the case of laying hens, there is also water lost in eggs. Surplus water must be removed in order to maintain osmotic balance. In a stable environment the only loss with a similar magnitude of variability to water intake is the excretory component (Hill *et al*, 1979).

2.6.1. Evaporative water loss The evaporative properties and high specific heat capacity of water make it an important regulator of body temperature. Evaporative water loss comprises respiratory and cutaneous loss. It is increased by raising ambient temperature, and, or, a lower relative humidity. On average, this route of water loss accounts for 17 % of total loss, within a range of 10 to 25 %, between 16 and 27 °C. Schmidt - Neilson *et al* (1969), stated that birds have no sweat glands, and that therefore cutaneous losses are negligible and evaporative losses almost entirely of respiratory origin. Respiratory loss occurs via the moist surface layer of the respiratory tract to the inspired air, which is saturated with water vapor at body temperature. Medway and Kare (1957) however, suggested that an appreciable amount of water may be lost by the cutaneous route, and Van Kampen (1974) who quantified evaporative loss, found that, between 10 and 30 °C, cutaneous loss represented approximately 40 % of total evaporative loss. Richards (1976) also

demonstrated that at the lower end of this temperature range, cutaneous losses appreciably exceed respiratory losses.

Various workers have given data for total evaporative water loss at temperatures around 20 °C. Barott and Pringle (1941) suggested 17 g/kg day, Van Kampen (1974) 12 g/kg day and Richards (1976) 28 g/kg day (determination by open flow technique) or 32 g/kg day (determination by direct weighing technique). However Hutchinson and Sykes (1953) have shown acclimation to reduce rate of evaporation. This was thought to be of adaptive significance as sustained evaporative cooling would greatly increase water requirement.

2.6.2. Urinary and faecal loss In the fowl both urine and faeces collect in the cloaca before expulsion. Water unabsorbed in the gut remains in the faeces and is passed from the body in excreta. The quantities of water excreted in faeces and urine, and therefore the excreta moisture, vary with differences in water intake. Most cases of raised excreta moisture are in laying hens, this being related to the greater requirement for water during egg formation (Medway and Kare, 1958; Leeson *et al,* 1995). There is considerable variation in estimates of the water content of excreta between workers. This is probably a result of variation in diet and ambient temperature, and therefore evaporation. Values suggested include 778 g/kg (Yushok and Bear, 1948), 650 g/kg to 782.7 g/kg (Hart and Essex, 1942), 850 g/kg (Jull, 1949), 800 g/kg (Anderson and Hill, 1968), 600-700 g/kg (Kerstens, 1964), 700 g/kg (Scott *et al,* 1982). Under normal circumstances for the laying hen, the quantity of water excreted in the faeces is about four times that excreted as urine. This loss will be subject to considerable variation with the amount and nature of undigesta, and the osmolarity of the urine component of the excreta (Leeson and Summers, 1992).

Many authors (e.g. Medway and Kare, 1959; Anderson and Hill, 1968; Osbaldiston, 1969; Lintern

- Moore, 1972) whose interests were in the relationship between the quantity of water drunk and that lost in excretion, did not attempt to differentiate between the water loss in faeces and urine. Also, with the exception of the first named authors, the others relied on making daily collections of the droppings, and weighing and drying them to constant weight. Since the rate of evaporation from freshly expelled excreta is high, estimates of the water content of droppings collected at intervals from open trays are inaccurate (Hill *et al,* 1979). Medway and Kare (1959) overcame the problems by collecting the droppings under oil, but introduced other inaccuracies since they estimated the water content of the droppings by subtracting the dry weight of the droppings of another group from the weight of the wet droppings of the experimental birds. Hill *et al* (1979) pointed out that there are sampling errors of inherent magnitude in this method. However, they concluded that the total water eliminated by 32 week old White Leghorns was 66 % of the water drunk. Dixon (1958) made both separate and combined assessments of the water content of urine and faeces, and confirmed this figure.

2.6.3. Water loss in eggs Draper (1966) determined water content of eggs in Brown Leghorn and Thornber 404 birds, and found mean values of 610 and 620 g/kg respectively whereas Favret *et al* (1967) reported values of 660 g/kg and Hill (1977) 683 ±38 g/kg for eggs from Shaver 288 pullets, and 689 ±39 g/kg for eggs from Warren SSL pullets. Hill *et al* (1979) pointed out that the magnitude of the standard deviation in the latter work was such that substantial errors could occur in calculation of a water balance, for an individual bird on a particular day, if the water content of the egg laid on that day was not measured directly.

2.7. PHYSIOLOGICAL CONTROL OF WATER EXCRETION

Water excretion is controlled by hormonal regulation of tubular reabsorption in the kidneys, and through reabsorption of urine water in the distal portion of the gut.

*2.7.1. **Tubular reabsorption*** Urine production is controlled by tubular resorption of water, the normal daily range for the hen being between 0.05 and 0.20 l/d. Following glomerular filtration fluid enters the renal tubules where it is isotonic with plasma. In the proximal tubules absorption of sodium ions and water occurs reducing the volume by 85 % under normal circumstances, but maintaining tonicity. As fluid passes to the distal tubules, via the loop of Henle, it becomes hypotonic through the reabsorption of sodium. The loop of Henle, associated with the vasa recta, the distal tubules and the collecting ducts form a concentrating system for urine (Kokko, 1977), allowing production of urine of an osmolarity higher than that of plasma. The descending limb of the loop of Henle is permeable to water and sodium; the ascending limb is far less permeable to water , but contains an active sodium pump, driving sodium into the extracellular fluid of the renal medulla. This countercurrent circulation generates a high gradient of osmotic pressure along the kidney papilla (Skadhauge and Schmidt - Nielson, 1967). In order to regulate the final urine tonicity, water can be reabsorbed in the last part of the distal tubules, and in the collecting ducts, due to the high osmotic pressure in the papilla, subject to the presence of antidiuretic hormone (ADH) which increases membrane permeability to water. Without this the urine becomes hypotonic. As little as 6 % of filtered water may be resorbed during water diuresis, and as much as 99 % at low rates of urine flow (Shoemaker, 1972). This can vary urine osmolarity between 700 and 40 mOsm and flow rate between 18 ul/ kg and 298 ul/kg. The concentrating capacity of the kidneys is limited by the amount of electrolytes and nitrogenous substances excreted (Skadhauge, 1973). Consequently if the tubular fluid flow rate were to increase due to excessive water

consumption, or the need to excrete an excess of solute, the maximum concentration of urine would fall and the volume increase. In the presence of ADH this would not be less than in plasma.

*2.7.2. **Cloacal reabsorption*** Many investigators have suggested that water from uretal urine is reabsorbed in the cloaca and rectum (Wiener, 1902; Das, 1931; Hester *et al*, 1940; Korr, 1939; Hart and Essex, 1942; Weyrauch and Roland, 1958; Sturkie and Joiner, 1959; Sturkie, 1965) and Clara (1926) stated that the lining of the coprodeum and urodeum, as well as the rectum, were suited to water reabsorption. These suggestions were based on observations of water requirements or increased urine flow, collected from cannulated ureters, or from the cloaca following plugging of the rectum. However, Hart and Essex (1942) suggested that although water is reabsorbed into the cloaca it does not occur in appreciable amounts, which suggests urine is secreted by the kidneys in a concentrated state, and Dixon (1958) indicated that little or no water was reabsorbed in the cloaca. Weyrauch and Roland (1958) attempted to measure water reabsorption in the cloaca of the chicken by introducing a solution containing tracer isotopes into the cloaca, and measuring the content of tracer isotope in the bloodstream. The figures indicated that 7.6 % of the isotope was absorbed within 4 h, but the studies failed to distinguish between cloacal absorption alone and that in the anus and gut. Roseler (1929) showed that, following caecectomy, the water contents of the droppings were increased as compared with unoperated birds, suggesting that the ceaca were involved with water absorption and data of Osbaldiston (1969) confirmed this report.

Akester *et al* (1967) and Koike and McFarland (1966) both using radioisotopes in intact birds, provided conclusive evidence that urine accumulated in the copradeum of the cloaca after flowing from the urodeum, where it was forced by antiperistaltic waves into the colon and large intestine, where reabsorption occurred. The osmotic and ionic compositions of the contents of the copradeum therefore, vary with the salt load and level of hydration of the bird. The varying

osmolarity and ionic concentrations of urine modify the composition of cloacal contents, and therefore the milieu of the mucosal side of the epithelial cells which in turn effects the transmural transport of solutes and water in this segment. The osmolarity of the contents of the copradeum therefore range from 489 mOsm, to 143 mOsm in the dehydrated and hydrated state respectively and in the large intestine from 376 mOsm to 192 mOsm (Skadhauge, 1968). Since the osmotic permeability coefficient is 3.2 ul/kg h mOsm in the serosa to mucosa direction, and 5.8 ul/kg h mOsm in the mucosa to serosa direction in normally hydrated birds a significant osmotic water flow may occur across the wall of the intestine. From the dehydrated state to the hydrated state, the rate of urine flow varies from an average of 18 ul/kg min to 298 ul/kg min. This function of the cloaca is essential for water balance in birds (Skadhauge, 1976). Water resorption is of the order of 25-30 ml/day for the adult fowl as long as urine is hypotonic (Dicker and Haslam, 1972). The fractional water absorption or loss across the epithelium of the copradeum and large intestine, is dependant on the rate of urine flow. Thus, in the hydrated state, only 2.3 % of the ureterally excreted water is reabsorbed in the cloaca. Also, sodium chloride absorption in the salt loaded state is only 2 % of the ureterally excreted load. Only in the dehydrated or salt depleted state is the cloacal transfer capacity for water and sodium chloride significant, compared with delivery from kidney. The absorption of water and electrolytes can also occur during dehydration, and salt loading, although less is absorbed in the latter two situations (Skadhauge, 1968) as there appears to be no means of keeping uretal urine from exposure to cloacal membranes and the large intestine across which the exchange of water and solutes may occur (Shoemaker, 1972). Skadhauge (1981) has shown however that only in severely dehydrated states is urine composition severely modified.

2.8. DAILY PATTERNS OF WATER INTAKE AND EXCRETION IN THE LAYING HEN

There is much evidence of a daily pattern of water intake in the hen. This is thought to be the result of a temporal relationship between feed and water intake, water intake and feed intake being closely related in mature birds (Wood - Gush and Horne, 1970; Hill, 1977) although not in immature birds (Siegal and Guhl, 1956). Consequently when Hill (1977) altered patterns of feed intake in hens, water intake closely followed. The formation of the egg involves formation and transportation of considerable quantities of protein and minerals across the wall of the oviduct, in part derived from increased food consumption. This increased demand for raw materials requires increased fluid ingestion, consequently daily patterns are thought to be associated with the different stages of egg formation. On days when neither egg laying or egg formation occurs Wood - Gush and Horne (1970) have observed, a morning peak, a steady rate throughout the day, and a decline prior to darkness in both water and feed intake. On ovulation days an afternoon increase in food intake was observed, although both Hughes (1972) and Howard (1975) found the afternoon peak to be unaffected by ovulation. Mongin and Sauveur (1974) found a peak in water consumption in laying hens at the end of the light period, and related this to the water requirement of plumping of the egg. A similar increase in feed intake, at this time, was observed by Morris and Taylor (1967) which was related to the need to satisfy the calcium requirement for shell formation, and Hill (1977) reported a peak in both. Howard (1975) suggested a gradual increase in water intake, from 12 hours before until about 2 hours before oviposition, followed by a sharp fall and an increase after lay. Lifshitz *et al* (1967), Ballard and Bieler (1969), Wood - Gush and Horne (1970) and Mongin and Sauveur (1974) have also observed decreases in water and food intake in the hour prior to oviposition, and suggested that this was related to increased nest searching behavior. The latter two workers also observed a clear peak in water consumption immediately

following egg laying. Nys *et al* (1976) however observed a different pattern of intake in birds lit continuously.

There are variations in urine flow in laying hens with the stage of egg formation. Mongin and Laccasagne (1967) observed a peak one hour before laying, associated with increased excretion of sodium and phosphorus, and Howard (1975) has shown urine volume to follow similar fluctuations to water intake, falling just prior to lay. The latter worker also observed, similar but inverted trends in plasma and urine osmolarity.

CHAPTER 3.

FACTORS EFFECTING WATER INTAKE AND EXCRETION IN POULTRY

3.1. INTRODUCTION

Hill (1977) pointed out that any factor which influences the value of the individual terms in the water balance equation (Equation 2.3.1) must influence the complete water balance. In order to maintain a balance, any factor which causes loss must be compensated for by an increase in one of the inputs, or a decrease in one of the other losses. The most common consequence of a disturbance in the water balance in poultry is raised excreta moisture, which is a problem in modern, highly mechanised laying houses, and a major factor in the accumulation of moisture in broiler house litter. Variation in excreta moisture occurs through an increased urinary, and, or faecal elimination of water, both being eliminated via the cloaca in the fowl (King and Mc Cleland, 1984). The quantities of water excreted in the faeces and urine depend upon the water intake. Factors which influence water intake and excretion in poultry fall into two classes, dietary and non dietary parameters. The effect of each class will be examined individually in the following chapter however, although all the factors discussed can have individual effects, it is often additive effects of several components which influence the water balance. Emphasis will be placed on water drunk and urinary and faecal loss of water throughout the chapter.

3.2. NON DIETARY FACTORS

3.2.1. Environmental temperature Differences in environmental temperature affect water consumption and excretion by the fowl. Increased environmental temperatures increase water intake in the short term (Wilson, 1948; Heywang, 1941; Hillerman and Wilson, 1955; Patrick, 1955; Ito *et al,* 1970; Snetsinger, 1973; Hamid and Sykes, 1979; Kechil *et al,* 1981; Bailey, 1990; May and Lott, 1992; Belay and Teeter, 1993) and in the longer term (Hillerman and Wilson, 1955; Ito *et al,* 1970; Parker *et al,* 1972; Smith, 1972). The initial large change in water consumption, which accompanies a sudden change in ambient temperature, is not maintained as

birds become acclimatised (Kechil *et al,* 1981). Despite a large increase in water intake, Hamid and Sykes (1979) have shown that it does not exceed 80 % of the water lost by evaporation. Consequently, in order to maintain water balance, other mechanisms must reduce water loss and the requirement for water, in order to prevent a water deficit. Following acclimatisation the rate of evaporation is reduced (Hutchison and Sykes, 1953). Kechil *et al* (1981) demonstrated that drinking occurs without any change in temperature of the body core and suggested that it was associated with a rise in temperature of the unfeathered skin, allowing an increase in water intake prior to any substantial loss of water. They went on to suggest that the effect was mediated by the rennin angiotensin system, which is activated by changes in distribution of extracellular fluid, the plasma rennin concentration increasing with environmental temperature in mammals.

Feed intake also decreases with increased environmental temperature, and layers consume twice as much water at 30 °C than 15 °C (Hillerman and Wilson, 1955) therefore the ratio of water to food ingested is increased at higher temperatures. Budgell (1970) described three hypotheses to explain the relationship between water intakes and environmental temperature, (1) stimulation of water intake at high temperatures due to local dryness of oropharyngeal receptors, (2) systemic dehydration, and (3) alteration in brain temperature.

At cold environmental temperatures, water intake may be reduced, although birds usually show little aversion to water as cold as 0 °C (Parker *et al,* 1972). Metabolic body water, created from increased fat metabolism, may be of significance in contributing to the body water pool at low environmental temperatures.

Van Kampen (1974) and Richards (1976) have both shown increased evaporative water loss with environmental temperature, and Vo *et al* (1978), Azahan and Sykes (1980), Van Kampen (1981), Teeter and Smith (1986), Lott (1991), Whitting *et al* (1991) and Belay and Teeter (1993) have

indicated considerable variation in urine output in response to increased environmental temperature. The latter showed that water excretion increased by 133 % when ambient temperature changed from 24 to 35 °C with *ad libitum* water consumption, but only by 64 % with restricted water intake. The authors concluded that the increased urine outputs were independent of water intake. Increased water excretion was largely accounted for by hypo-osmotic urine with increased osmolar and free water clearance, as also shown by Van Kampen (1981). Azahan and Sykes (1980) however showed that raised ambient temperature (29 and 32 °C), reduced urine flow and increased osmolarity, whereas at 40 °C and above, and at 0 and -5 °C, urine flow increased and osmolarity decreased. It was concluded that the anti-diuresis seen at warm ambient temperatures was caused by the release of antidiuretic hormone, possibly as a result of a rise in skin temperature, as hypothalamic heating had no effect. This contrasts with diuresis seen at low environmental temperature which can be induced by cooling of the hypothalamus in the duck (Simon - Oppermann, *et al* 1979).

3.2.2. Age For all poultry, water intakes and the ratio of water to feed intakes, as well as water excretion, increase with age (Witter, 1936; Medway and Kare, 1959), although consumption per unit body weight decreases. This is reflected in the body water values which Lopez *et al* (1973) found to be 57 and 76 % of body weight for seven year old hens and five month old pullets respectively. For growing pullets, water intakes increase gradually, but decreases from 0.45 g/g body weight at 1 week of age to 0.13 at 16 weeks of age (Medway and Kare, 1959). A sudden increase in intake occurs, associated with sexual maturation, but once peak egg production has passed no obvious relationships between water consumption and age exist (Hill, 1977). Lumijarvi and Hill (1968) related the increased water consumption associated with egg production and maturity to the circulating oestrogen level. Linear increases in daily water intake with age have also been observed in broilers (Patrick and Ferrise, 1962; Kellerup *et al,* 1965; Pesti *et al,* 1985; Brake *et al* 1992) and Ross *et al* (1954) found that the water consumption was unaffected by

chick growth rate.

The water content of the body is associated with the protein content. As the bird ages its body fat content increases, and therefore the body water content and intake, as percentages of body weight decrease, although Lopez *et al* (1973) suggested that a real decrease in intra and extracellular body water may occur with age. Medway and Kare (1957) reported evaporative water losses from white leghorn pullets at various stages of growth. They found that evaporative water loss was quite high for the first day after hatching, followed by a dimunition for two weeks after which it rose again, and levelled off to the level of an adult. In young birds a higher percentage of water is eliminated through respiration, but, as weight increases, a higher percentage is eliminated through the droppings.

3.2.3. Food intake Food intake affects all water inputs, ie metabolic water, the water content of the food, and water drunk. Evidence for the latter is provided by strong correlations between food and water intake, and a similarity in temporal distribution of feeding and drinking. Factors which affect feed intake will also affect water intake (Bierer *et al,* 1966; Zeigler *et al,* 1972). Under *ad libitum* conditions, water intake is related directly to food eaten (Dixon, 1958; Patrick and Ferrise, 1962; Ibarbia, 1968). Although Tyler, (1958) could detect no relationship between hourly food and water intake, others (Hill *et al,* 1979; Savory, 1978; Wood - Gush and Horne, 1970) have all shown hourly feed consumption to correspond closely with hourly water consumption. There is however no fixed ratio between feed and water consumption (Bailey, 1990) as it is subject to variation, depending on dietary components and environmental conditions. However using similar experimental conditions with laying hens both Van Kampen (1983) and Savory (1978) have suggested that for every 5 g increase in feed consumption there is an 8.5 g increase in water consumption. The significance of the association is thought to be in the maintenance of water balance (Bailey, 1990). Van Kampen (1983) with layers, and Marks (1981; 1985) with broilers

have demonstrated that birds with a high water: feed ratio have an improved feed utilisation.

*3.2.4. **Water temperature*** The temperature of water has a direct effect on the water intake, although the response is also dependant on environmental temperature. Wilson and Edwards (1953) found that birds at 32 °C drank more cold water than water at room temperature, although the response was not statistically significant. In contrast both Medway and Kare (1958) and Herrick, 1971) found that birds drank more water in winter when the water was warmed, although the former stated that warming beyond 10-13 °C caused them to drink less. Medway and Kare (1958) found that intake was reduced noticeably when water temperature reached 41 °C and that birds refused to drink when it reached 44.5 °C. Similarly Gates and Kare (1961) and Prince and Kare (1962) demonstrated a decline in intake with increased temperature. However in the former work, the point at which complete rejection occurred was dependant on ambient temperature. It is possible, therefore, that water temperature *per se* may limit water intake at high environmental temperature. Despite these trends, the response to water temperature has been shown to be variable between birds when used to generate a response in the lingual nerve (Halpern, 1962).

*3.2.5. **Egg production*** Large changes in water intake, and water to feed intake ratio, occur at the onset of sexual maturity associated with egg formation and transportation of protein and minerals across the wall of the oviduct. Various workers (Medway and Kare, 1958; Medway and Kare, 1959; Anderson and Hill, 1968; Van Kampen, 1974) have noted that the ratio of water drunk to feed consumed is greater in laying hens than non laying hens, and Jull (1949) and Heywang (1941) suggested the daily water intake to be greater as the number of eggs per year increased for a laying flock, although Hill (1977) disputed this. Anderson and Hill (1968) reported water to feed ratios of 1.21 ±0.02 g water per g of feed during pre lay, 2.04±0.20 g/g during the laying period, and 1.33±0.20 g/g during the post lay period. Howard (1975) found the number of drinks taken on egg laying days to be higher, but the size to remain constant, so that total intake increased. Van

Kampen (1974) also found the water to food ratio to be higher on egg laying than non egg laying days, and Wood - Gush and Horne (1970) observed that total daily water intake was higher on days when laying occurred with no following ovulation, which suggests water requirements of egg formation contribute to the daily variation in water intake of individual birds. Howard (1975) found water output to be higher on egg laying days. The body water flux of the laying bird was found to be greater than both the adult male bird (Chapman and Black, 1967) and the non laying bird, even when the water contents of the egg and the urine were allowed for (Chapman and Mihai, 1972). The difference between the laying and non laying hen may relate to an increased reservoir of soluble metabolites necessary for egg production.

*3.2.6. **Hormone balance*** Brown *et al* (1958) reported that surgical trauma, the injection of deoxycorticosterone, cortisone acetate or ACTH caused an increased water elimination, but only deoxycorticosterone caused an increased water intake. In contrast adrenolectomy of cockerels caused a decreased water intake. It was concluded that the elimination of water in each case was probably the result of protein catabolism. This could suggest a relationship between trauma and water excretion. A reduction in anti diuretic hormone production could dispose birds to increased water excretion, however Osbaldiston (1969) suggested that wet droppings in hens were not likely to result from physiological suppression of avian antidiuretic hormone, as hens failed to respond to massive pitressin administration. Denbow (1985) found that intra- cerebroventricular injections of angiotensin II to increase water intake in a dose dependant manner.

3.2.7. Sex The differences between the sexes in water intakes and excretion cannot be attributed solely to egg production. Marks and Washburn (1983) found male broilers to consume more water than females, and Marks (1985) demonstrated that the divergence in water intake between

the sexes started immediately after hatching. Divergence in body weight also begins at this time and probably explains the observed differences in both feed and water intake between the sexes. The trend in mature fowls however differs from that of broilers. Lifschitz *et al* (1967) noted differences in water ingestion by male and female White Leghorn laying hens. Females consumed about 1.7 times more water than cocks. The observations were not confined to volume of water consumed, for the pattern of intake throughout the day also varied. The differences could not be attributed to egg production as reflected by water turnover data of Chapman and Mihai (1972) who found non laying hens to turn over a significantly larger fraction of their water pool than cocks.

3.2.8. Strain differences Hillerman and Wilson (1955) noticed differences in water intakes between White Leghorns, Jungle Fowl and a group of heavy breeds consisting of New Hampshires and Barred Rocks. Malik (1965) also showed significant differences in water intakes, and water excretion of three commercial laying stocks of birds, which characteristically were observed to produce dry, medium dry, or wet excreta. The differences reflected variation in non excretory water, and were suggested to be genetically linked. Data on water consumption and excretion of two inbred lines and their F1 crosses were also obtained. There were significant differences between the two lines, and both parameters were greater in the F1 cross than in either of the parental lines, suggesting heterosis for the traits. Buss and Murphey (1965) suggested excessive water intake to be the expression of an autosomal recessive gene. Ogungi *et al* (1983) compared water intakes of two strains of male broiler breeder, noted for differences in the looseness of their droppings. Significant strain differences were again found. Marks and Baik (1994) found in two random bred control populations following selection for high eight week body weight, under high (1.6 %), low (0.20 %) and normal (0.40 %) dietary salt selection environments, significant differences between genotypes, in water intake and water to feed intake ratio. In contrast to the above works, Hill (1977) found no differences between water consumption of a heavy bodied

strain of layers (Warren SSL) and a light bodied strain (Shaver 288) maintained under similar conditions. Braun and Stallone (1989) suggested that the reason why some strains consume large quantities of water, and produce wet excreta, was due to nephrogenic diabetes insipidus (an inability of the kidney to concentrate urine).

3.2.9. Drinker type The way in which water is presented to a bird can influence the amount consumed. Layers drink more water from troughs than from nipple drinkers (Hearn, 1976), as do broilers (Mc Masters *et al,* 1971). Bray and Lynn (1986) have also shown that small cups or nipple drinkers can reduce water intake as well as wastage in broilers when compared to traditional bell drinkers.

3.2.10. Behaviour Hungerford (1969) and Lintern - Moore (1972) both suggested that water intake of layers in cages may be greater as a result of boredom.

3.2.11. Housing type Yoselswitz (1991) stated that laying hens in cages drink more water than when they are kept on a litter floor. Hot temperatures often experienced in cage systems cause water intake to increase rapidly, which causes more moisture to be eliminated in the excreta.

3.2.12. Infectious agents This section is not intended as a review of all literature concerning the effect of infectious agents on water balance, but rather as a summary of the more important aspects. The majority of the information, unless otherwise specified, is drawn from Pattison (1989).

3.2.12.1. Bacteria *Escherichia coli* may cause diarrhoea. This is a result of infected birds drinking

more water rather than an effect of the *E. coli per se* on the gut mucosa, water consumption increasing if heavily contaminated with bacteria. *Campylobacter jejuni,* is a common organism in the intestinal tract of broilers aged two weeks or more (Neill *et al,* 1984). Isolation appears to often coincide with appearance of wet litter, although it has also been isolated from flocks without wet litter, and therefore may be strain dependant.

3.2.12.2. Protozoa Coccidia species *Eimeria* especially *E. necatrix, E. brunetti, E. acervulina* and *E. tenella* may cause enteritis and diarrhoea. Carbo - Baptista *et al* (1976) indicated that birds infected with coccidia often exhibit net water loss in the duodenum and mid lower jejenum. Broilers are at less risk than replacement breeders and pullets, as high levels of coccidiostat can be fed throughout their short lives. However, although control of coccidiosis is good with the widespread use of ionophore coccidiostats, controversy exists concerning the relationship between water balance and certain ionophore products. Lasolocid has been associated with wet litter relative to monensin (Ward and Brewer, 1981; Wheelhouse *et al,* 1985), possible due to the ionophores influence on acid base relationships and osmotic balance. In contrast, birds fed monensin have been shown to produce drier manure than birds not fed a coccidiostat and have depressed daily water intakes (Frigg and Broz, 1983), although Lehel *et al* (1995) could detect no effect of monensin on water intake. Salinomycin has been shown to be intermediate between monensin and lasalocid whereas narasin is similar to monensin in its effect on water balance (Nguyen, 1991)

3.2.12.3. Viruses Several viruses have been implicated as causes of diarrhoea (Mc Ferran *et al,* 1983) (Appendix 12). Mc Nulty *et al* (1984) showed that a crude inoculum of intestine containing enterovirus (associated with stunting syndrome in broilers) as well as reovirus infection would

produce faecal changes (faeces becoming mushy) and cause birds to congregate around drinkers. However reovirus alone could not produce these changes, and therefore enterovirus was suggested to be the important organism. Stunting syndrome has been associated with other viruses (Wyeth *et al* 1981; Farmer and Taylor, 1985 and Frazier *et al,* 1986) and it is likely that it represents more than one syndrome. Griffiths and Williams (1985) have described the runting as a temporary maldigestion of food over the first four weeks of life and Frazier *et al* (1986) found the pancreatic duct to be obstructed in stunted birds, leading to pancreatic atrophy and malabsorption. The digestive tract cannot absorb complex fats and carbohydrates, but still absorbs sugars such as glucose and xylose thus increasing the amount of starch and fat in the faeces and producing droppings of a yellowish colour which are both sticky and bulky. Infectious bronchitis may affect both excreta moisture and water intakes through kidney disfunction (Wideman and Satnick, 1989; Glahn *et al,* 1989 b) water loss most likely relating to increased sodium loss due to nephritis.

3.2.12.4. Mycotoxins Nephrotoxic mycotoxins such as ochratoxin and citrinin produced by the common moulds *aspergillus, ochraceus,* and *penicillium viridicatum* acutely increase sodium excretion, water consumption, increase urine flow, and manure moisture, although they do not significantly alter glomerular filtration rate (Huff *et al,* 1975; Ames *et al,* 1976; Krough *et al,* 1976 a & b; Roberts and Mora, 1978; Elling, 1979; Nelson *et al,* 1980; Page *et al,* 1980; Gustavson *et al,* 1981; Mehdi *et al,* 1981; Hnatow and Wideman, 1985; Glahn and Wideman, 1987; Glahn *et al,* 1988 a, b and c, 1989 a and b). These toxins are commonly found in home produced grains, but it is unlikely that they are often present at high enough levels to cause a problem.

3.2.13. Water treatment There has been an interest in the treatment of water for poultry, to prevent mineral deposition in pipelines. Normal treatment involves orthophosphate which

sequester calcium and magnesium, therefore preventing precipitation in the water supply. However as a last resort some producers use water softeners. Hard water is softened by an ion exchange system in which calcium and magnesium ions are replaced with sodium ions, thereby elevating sodium ion content in drinking water, and elevating water consumption (Roush and Mylet, 1986). The problem with water softeners depends on the area, as the amount of sodium pumped into the water is proportional to the water hardness (Leeson and Summers, 1992).

3.3. DIETARY FACTORS

Numerous nutrient and non nutrient components, as well as the physical characteristics of the diet have been related to water balance of poultry. In general increasing the concentration of these dietary constituents above dietary recommended requirement has been shown to increase water consumption. Much of the previously published work carried out, particularly earlier work, focussed not on the effect of individual nutrients but on the effect of dietary ingredients. As the observed physiological response to concentrations of these ingredients were the result of variation in nutrient composition of the diet they will be discussed under the relevant nutrient sub-heading.

3.3.1. Minerals Requirements for the macro - minerals have been defined in publications sponsored by the Agricultural Research Council (1975) and National Research Council (1984) (Appendix 1). If the dietary concentrations of the macro - minerals deviate from recommended requirements in a diet then they in general encourage increased water intakes and urine outputs in order to excrete the increased mineral loads via the urine. In addition, increased urine osmolarity, and osmolarity of undigesta will reduce water reabsorption from the distal gut (Rice and Skadhauge, 1982). Variation in water output will in turn vary the consistency of droppings. Diets are subject to variation in macro - mineral profile due to formulation mistakes, separation of mineral supplements from the bulk of the diet and variation in composition of dietary

components with source. Few feed manufacturers analyse each batch of ingredients for use in formulation. Feedstuffs that have not been processed tend to be consistent with regard to macro - mineral content. Further processing tends to generate greater mineral variability. Feedstuffs which represent bye- products of food processing poses least uniformity (Austic and Patience, 1988).

3.3.1.1. Dietary sodium - requirements Dietary raw materials destined for poultry are significantly deficient in sodium and supplementation is therefore necessary. Sodium is generally supplied in the diet as its chloride salt, although use of the bicarbonate and sulphate salts are also practiced. As a consequence, increasing dietary sodium concentration, by way of sodium chloride, will also normally increase dietary chloride concentration. The National Research Council (1984) state that the requirement for sodium is 1.5 g/kg of diet, regardless of age of the bird, and for chloride 0.8 g/kg of diet, and the Agricultural Research Council (1975) suggest 1.0 g/kg and 0.90 g/kg respectively. Sodium chloride does not provide the correct ratio of sodium and chloride at the desired levels, and therefore the anion - cation ratio may be affected by increased dietary concentrations.

3.3.1.1.1. Dietary sodium and water balance Much of the work concerning the effect of sodium on water balance describes the effect of salt, rather than attributing the response to either the sodium or chloride component. Concentrations of salt in the diet and water supply, in excess of recommended requirements increases water intakes of birds, the water to feed intake ratios, and the quantity of moisture in the excreta, and may predispose broilers to wet litter (Collier, 1892; Halpin *et al,* 1936 b; Selye, 1943; Barlow *et al,* 1948; Kare and Bielly, 1948; Heuser, 1952; Krista *et al,* 1961; Kando and Ross, 1962 b; Hurwitz *et al,* 1973; Hijikuro, 1976; Lee and Campbell, 1983; Marks and Washburn, 1983; Damron *et al,* 1984; Damron and Kelly, 1987; Smith and Teeter, 1989; Davison and Wideman, 1992; Marks and Baik, 1994; Spais *et al,* 1994; Leeson *et al,* 1995). Many of these workers and Ogungi *et al* (1983) who were only concerned

with sodium chloride levels between 0 and 7 g/kg suggested, that the response was not observed in birds consuming diets containing less than 10 g/kg of salt, although Lee and Campbell (1983) fed roosters 4 and 10 g/kg dietary salt levels and found both increases in water consumption and excreta moisture, with increases in sodium concentration in corn based diets, although not in rye based diets, (possibly as a result of differing salt utilisation in high dietary fibre, rye based diets). Damron *et al* (1984) also stated that in broilers fed dietary sodium chloride concentrations ranging up to 17.5 g/kg, water intake and excreta moisture were most sensitive between 0.4 and 2.5 g/kg supplemental sodium chloride. Patterson *et al* (1989) in layers, also reported that whereas sodium chloride concentrations between 1.5 and 7.5 g/kg had no influence on water intake, the excreta moisture was increased at the 7.5 g/kg concentration. Water balance rapidly returns to normal following replacement of diets containing excess salt as shown by Damron and Kelly (1987) on switchback from a 60 g/kg to a 4 g/kg sodium chloride diet. In contrast to the above data Anderson (1968) fed diets containing 0.3, 0.7 and 1 g/kg sodium, mainly as sodium chloride, to nineteen week old shaver pullets and found the water to feed intake ratio, and excreta moisture of the birds, to be greater in those birds fed the low sodium diet than for the other two groups. Lumijarvi *et al* (1967) also reported that in colostomised birds water intake and urine output were markedly increased when salt intake was restricted. It is likely that the effects of salt deprivation on water balance of the colostomised fowl are more readily induced than in the intact fowl.

Sodium is the principle osmotically active electrolyte in plasma and urine. It is actively absorbed in the intestine, although less efficiently (41-48 %) than in mammals (90 %), and carried to the kidney where it may be re-absorbed into plasma or excreted in urine. Consumption of excess salt will raise plasma sodium ion concentration, which must be excreted to maintain osmotic potential and electrolyte balance of the body. Consequently increased urinary sodium excretion, glomerular filtration rate, and urine flow occur with sodium excess, except at very high salt loads, when glomerular filtration and urine flow decrease (Dantzler, 1966; Martindale, 1975; Wideman *et al*

1987; Wideman, 1988; Vena *et al,* 1990). Davison and Wideman (1992) found when feeding excess sodium bicarbonate that the osmotic effect of increased sodium excretion increased the fraction of filtered water excreted, producing wet excreta and therefore increasing water intake. However the wet excreta could not be attributed entirely to increased urine flow, as birds on the high sodium diet had significantly lower absolute urine flow rates. Under normal conditions substantial quantities of water and sodium chloride from ureteral urine are reabsorbed from the lower intestine of poultry. This process is inhibited when hens are fed diets containing 10g/kg sodium chloride (Rice and Skadhauge, 1982).The relatively low absorption of sodium may allow unabsorbed sodium to influence water reabsorption in the hindgut at higher concentrations of sodium intake. Hurwitz *et al* (1973) found when feeding two concentrations of sodium 0.7 and 1.5 g/kg that apparent absorption was only increased from 41 % to 45 %.

3.3.1.1.2. The effect of the sodium anion The effect of salt on water balance could represent a response to either sodium or chloride, a combined response, or a change in the ratio of the two minerals. It has been suggested that moisture content of excreta may be reduced by replacing sodium chloride for bicarbonate. The effects are however equivocal. Raised water consumption and watery droppings have been observed in birds fed excess sodium as sodium bicarbonate (Witter, 1936 in chicks; Davison and Wideman, 1992 in layers) and when supplied in the water between 5 and 34 g/l (Witter, 1936; Christopher, 1977). Damron *et al* (1984) found water intake to be higher for sodium bicarbonate than chloride fed broilers at equivalent sodium concentrations and could detect no difference in excreta moisture between the two sources. Also Damron (1982) supplied 0.4 g/kg sodium as either sodium acetate, sodium sulphate or sodium bicarbonate, and found all sources to increase daily water intake and excreta moisture to similar extents, when equivalent concentrations of sodium were fed, the response to sodium sulphate being confirmed by Adams *et al* (1975) although Cuervo (1972 b) could find no effect of sodium sulphate on excreta moisture. With exception of the latter work, these reports all suggest that it is sodium itself

rather than its counterion which act on water balance. Both Kando and Ross (1962 a) and Vogt (1971) supported this, the latter showing chloride to have no effect on water content of excreta in broilers at 2 or 5.1 g/kg regardless of sodium concentration. In contrast however Leeson and Summers (1992) cite trials in layers where substitution of dietary supplementary sodium chloride with bicarbonate, at 30 % of the supplemental concentration, is beneficial in reducing excreta moisture without loss of production, although they emphasised the need to balance chloride. In a second study sodium bicarbonate was added to layer rations without reducing sodium chloride. There were no problems of wet droppings suggesting the two sodium sources were non additive.

3.3.1.2. Dietary potassium - requirements Potassium plays an important role in the homeostasis of the body fluids. Potassium concentration in plant material is high. Most carbohydrate and protein sources used in poultry diets are therefore rich in potassium. Dietary concentrations are therefore often far in excess of the nutrient requirement which the National Research Council (1984) suggests to be 1.5 g/kg and the Agricultural Research Council (1975) 2.5 g/kg, and are rarely controlled in practical diets. Concentrations are therefore subject to considerable variation.

3.3.1.2.1. Dietary potassium and water balance Potassium in the diet in excess of the recommended nutrient requirements, increases both the water intakes of birds, the water to feed intake ratios, and the quantity of moisture in the excreta, and may predispose broilers to wet litter. The majority of work in which these effects have been observed has focused on feeding incremental levels of molasses, in which potassium concentrations are high, to chickens (Winter, 1929; Bice and Dean, 1939; Rosenburg and Palofax, 1956; Kando and Ross, 1962 a and b; Cuervo *et al,* 1972 a and b; Jordan, 1990; Walker *et al,* 1994) and to mature birds (Rosenburg, 1955; Rosenburg and Palofax, 1956) although the sugar constituent of molasses may also be a contributer (Kando and Ross, 1962 a). Rosenburg (1955) suggested de-ionisation to reduce the effect of molasses on excreta moisture and Kando and Ross (1962 a) fed partially de-ionised

molasses and found reductions in the moisture content of the excreta, following de-ionisation although Cuervo *et al* (1972 b) could find no benefit. Others (Kando and Ross, 1962 a and b; Cuervo *et al,* 1972 a; Vogt, 1971; Smith *et al,* 1973; Frigg and Broz, 1983; Hijikuro, 1976) fed diets containing excess potassium as the inorganic salt. They also found strong correlations between excreta moisture, water to feed intake ratios and water intakes and the potassium concentration of the feed, and Hijikuro (1976) found a correlation between excreta moisture and potassium concentration of the excreta. Potassium chloride in solution has also been shown to increase water intake of broilers (Teeter and Smith, 1986; Smith and Teeter, 1987; Smith and Teeter, 1989; Beelay and Teeter, 1993) the latter workers showing a 91 % increase in water intake with 0.75 % potassium chloride supplementation.

Kando and Ross (1962 a) fed diets containing 27.3 g/kg and 35.8 g/kg of potassium as either the chloride or the carbonate salt. There were no differences in excreta moisture level between the two inorganic sources but in a comparison between equivalent concentrations of potassium as molasses and inorganic salts, the response was greater for the inorganic form, suggesting some potassium in molasses to be bound in inaccessible form.

Whittemore *et al* (1975) have shown in two experiments that, as the level of cooked potato in the diet increases, the litter moisture in broiler systems increases. In the second of the two experiments, litter dry matter fell to 260 g/kg at 50 days of age, at a 400 g/kg dietary concentration. Wet excreta was also noted as characteristic of potato feeding by Vogt (1969 a and b) and Vogt and Stute (1969 a and b) and the latter workers implicated potassium as the causative factor, potato containing approximately 20 g/kg of potassium, although the high water absorbing capacity of potato containing diets might also induce birds to drink and excrete more water.

Brown and McCracken (1965) recorded an apparent absorption value of 73 % for potassium in

layers, whereas Hurwitz *et al* (1973) reported 83 and 76 % depending on sodium concentration. As the amount of potassium ingested increases, the amount of potassium retained and excreted increases (Kando and Ross, 1962 a). Potassium is essentially an intracellular element, involved in regulation of fluid volume and acid base balance within the cell, in contrast to the extracellular localisation of sodium. Potassium loads are consequently rapidly pumped into cells with parallel migration of water. The extracellular volume decreases and its osmolarity increases, which increases water intake, glomerular filtration rate and tubular secretion and results in excretion of a potassium rich urine. As the concentrating capacities of the kidneys are limited, urine volume is dependant on amount of solute for excretion, and therefore increases with potassium concentration.

3.3.1.3. Dietary calcium and phosphorus - requirements Calcium requirements are concerned principally with production, as maintenance needs are small. Laying hens need large amounts of calcium in their diet, as the mineral matter of egg shell comprises almost 40 % calcium. The Agricultural Research Council (1975) recommendations are for 35 g/kg with an average feed intake of 110 g/b/day whereas the National Research Council (1984) states the requirement to be 32.5 g/kg of the diet. Non laying birds and broiler chickens require less calcium (between 6 and 10 g/kg depending on age). Phosphorus plays a fundamental role in both the structure, and cellular function, of living organisms. Although inorganic sources of phosphorus have high availability, the intestinal release of phosphorus from organic molecules is variable. The most important phosphorus requirement are, as with calcium, associated with production. Phosphorus requirements of the laying hen are considerably lower than those for calcium, the egg shell containing very little phosphate. The National Research Council (1984) state that the requirement for available phosphorus to be 5.0 g/kg whereas the Agricultural Research Council (1975) state 3.50 g/kg.

3.3.1.3.1. Dietary calcium and water balance Unlike potassium and sodium Glahn *et al* (1988 c) and Wideman *et al* (1989) state that calcium excess does not increase water consumption or excreta moisture and Quissenberry and Walker (1970) have reported that oyster shell addition to the diet can reduce excreta moisture in layers. Keshavarz (1991) found that excreta moisture was reduced by increasing the proportion of supplemental calcium as calcium carbonate for gypsum (calcium sulphate). However Shaffey and Mc Donald (1990) with sexed growing chicks found excess dietary concentrations of calcium (25.50 and 33.10 g/kg) to increase both plasma calcium concentration and excreta moisture. Interactions between dietary calcium and available phosphorus were observed as well as calcium x sex interactions. Roland and Caldwell (1985) and Leeson and Summers (1987) have both demonstrated an apparent relationship between raised excreta moisture and low concentrations of dietary calcium in mature laying hens. Roland and Caldwell (1985) fed calcium ranging from 0.5 g/kg to 37.6 g/kg to hens. Excreta of birds fed 0.5 g/kg became extremely watery compared to that of controls, but was not related to increased water intake, but a decreased feed intake which increased the water to feed intake ratio. Jensen (1977) observed a similar response in broilers. Birds fed diets containing 6 g/kg calcium had raised excreta moisture and wet litter that disappeared within three days after increasing dietary calcium to 10 g/kg.

The mechanism responsible for watery excreta from hens when fed inadequate calcium is unknown. Roland and Caldwell (1985) cited Cuthberts (1970) observation that calcium is involved in release of several hormones, including vasopressin, but also pointed out that Osbaldiston (1969) has suggested that wet droppings in hens are unlikely to result from physiological suppression of avian antidiuretic hormone, hens failing to respond to massive pitressin administration. Within normal dietary limits the requisite level of ionised blood calcium is maintained in the laying hen by ionic calcium absorbed (via calcium binding protein level) in the intestine; calcium protein blood serum complexes capable of dissociating; surface active

calcium reserves in the skeleton and calcium liberated during bone resorption all under the control of parathyroid hormone and vitamin D (Georgievskii *et al,* 1990). It is therefore not surprising that excess calcium has little effect on water balance in the laying fowl and that differences between laying and non laying hens exist.

*3.3.1.3.2. **Pre-lay calcium concentrations and water balance*** Leeson *et al* (1987) related early introduction of layer diets, prior to maturity, containing appreciable concentrations of calcium (35-45 g/kg) to excessive water intake and raised excreta moisture. Although favourable in terms of medullary bone deposition, as more calcium is retained when high concentrations are fed, the relationship is not linear and so increased quantities of calcium are excreted, along with increased water excretion. While particularly problematic in the immediate pre-lay period, such treatment may also have long term effects on excreta moisture. Pre-lay diets which contain intermediate (20 g/kg) concentrations of calcium allow this problem to be overcome (Leeson *et al,* 1995). The mechanism of this response to calcium excess is poorly understood. Elevated dietary calcium can produce polydipsia and polyuria as a result of hypercalceamia in mammals although once it is corrected then polyuria is prevented. Hypercalceamia is known to stimulate calcitonin, a peptide hormone from the thyroid, which inhibits osteolysis, and increases renal excretion of calcium, phosphate and other minerals and could cause polyuria and polydipsia. Foorman and Leeson (1959) also suggested excess calcium may lead to stimulation of thirst centres in mammalian brain, although any such relationship in avian species is unknown.

*3.3.1.3.3. **Dietary phosphorus and water balance*** There is little information on the effect of dietary available phosphorus on water balance. However Shaffey and Mc Donald (1990) in growing chicks indicated that high dietary available phosphorus concentrations increased excreta moisture.

3.3.1.4. Dietary magnesium - requirements Magnesium is present predominantly within cells, where it is involved in reactions based on ATP. It is absorbed actively and excreted by the kidney. However the kidney has considerable capacity for magnesium reabsorption and therefore maintenance requirements are extremely small. The amount of magnesium in feedstuffs is therefore normally more than adequate to meet the need of birds. The risk therefore, in practical situations, is an excess in the diet. The National Research Council (1984) suggest the requirement to be 0.6 g/kg for layers and 0.5 g/kg for broilers and these levels are a little higher than those of the Agricultural Research Council (1975) of 0.4 and 0.45 g/kg respectively.

3.3.1.4.1. Dietary magnesium and water balance The effects of magnesium on water balance of the bird are generally seen when feeding dolomitic limestone. Dolomitic limestone is a good source of calcium, containing 200 g/kg calcium, but unlike conventional limestone (10 g/kg) it contains 120 g/kg magnesium. Numerous workers have reported increased water excretion as a result of increased magnesium concentration of the diet, either through feeding dolomitic limestone as free choice grit (Alder, 1927) or in replacement for other calcium supplements, (Tully and Franke, 1934; Stilmak and Sunde, 1971) or by increasing the ratio of magnesium carbonate to calcium carbonate in the calcium supplement (Supplee, 1963; Mehring, 1965; (chicks), Mehring and Johnson, 1965; Mc Ward, 1967; Stilmak and Sunde, 1971 (layers)). The increased excretion of water is associated with excessive drinking (Leeson *et al,* 1995) and occurs as birds attempt to clear the mineral through the kidney, although Adams *et al* (1975) found that increased concentrations of magnesium sulphate decreased water consumption and neither Kando and Ross (1962 a) or Cuervo *et al,* (1972 b) found any effect of either supplementary magnesium oxide or sulphate on excreta moisture in chicks.

Older birds appears more tolerant to magnesium (Atteh and Leeson, 1983), which is reflected in the water balance. Mehring and Johnson (1965) found that concentrations of magnesium from

5.65 g/kg to 7.33 g/kg did not produce wetter excreta in layers, although there was a significant increase between 8.37 and 11.52 g/kg whereas Mehring (1965) in chicks found that concentrations of magnesium ranging from 5.65 g/kg to 6.80 g/kg raised the excreta moisture. Data of Mc Ward (1967) in layers supported this as rations which contained 4.8 or 7.0 g/kg magnesium produced little sign of damp litter. However much damper litter resulted from the raised excreta moisture of hens which consumed rations with 12.0 and 19.6 g/kg magnesium.

The effect of magnesium could depend on the source. Stilmak and Sunde (1971) have shown that dolomite will increase moisture content of litter of birds to a lesser extent than magnesium carbonate even though the amount of magnesium it could supply was potentially greater.

3.3.1.5. Trace elements and water balance High concentrations of dietary copper have been reported to reduce excreta moisture in turkeys (Leeson *et al,* 1995) although Jensen *et al* (1978) has shown 120 to 150 mg/kg additional copper to give darker and more pasty excreta in broilers. Sturkie (1956) investigated the effect of water soluble zinc sulphate in drinking water on water consumption of White Leghorns for twenty days. Water consumption decreased rapidly after 24 h. However after 365 h birds had developed a certain tolerance, although consumption was still half normal. The decrease in water intake seemed to be caused by birds finding zinc distasteful since they drank readily at first but later realised the water was toxic. The practical relevance of these observations, which used concentrations far in excess of those normally found, was however questioned by Bailey (1990).

3.3.1.6. Macro - mineral interactions and water metabolism In many species sodium and potassium have been shown to be antagonistic to one another in their affect on water balance (e.g. Ray and Talapatra, 1945). However in poultry, dietary sodium and potassium have been found to be additive on both water intake and the moisture content of excreta of broilers (Kando and

Ross, 1962 b; Vogt, 1971) and pullets (Hijikuro, 1976) as well as on urine excretion of pullets (Hijikuro, 1976) when in dietary excess. Although water consumption, excreta moisture and urine output all increased with increased potassium concentration regardless of dietary sodium concentration, the main effect in each study was due to sodium. Vogt (1971) has shown that although sodium and potassium are additive neither sodium and chloride or potassium and chloride are. Chloride could however not be shown to reduce the response to sodium or potassium in the latter work, although Hurwitz *et al* (1973) showed that dietary chloride supplementation suppressed the moisture content of excreta when added to diets with a high sodium to chloride ratio, although little reduction occurred, with a ratio of 1: 1 or lower. Both Kando and Ross (1962 b) and Hijikuro (1976) have suggested that excreta moisture may reach a point at which neither an increase in potassium or sodium concentration could increase it further.

Kando and Ross (1962 b) have shown that increased dietary calcium concentrations (10- 30 g/kg) in diets high in potassium (10 g/kg) tend to decrease ($p<0.05$) excreta moisture of broilers although no effect was observed in rations containing 5 g/kg or less potassium. The antagonistic effect agrees with data from other species (Pfeifer *et al,* 1941; Merril *et al,* 1951).

3.3.2. Dietary crude protein - requirements The National Research Council (1984) suggests the requirement for crude protein of the laying hen to be 150 g/kg of the diet and the Agricultural Research Council (1975) 165 g/kg. In contrast the requirements for the broiler are given as 230, 200 and 180 g/kg at 0-3, 3-6 and 6-8 weeks respectively by the former. However, birds do not have a requirement for crude protein *per se,* but for individual amino acids although there should be sufficient to ensure an adequate nitrogen supply for synthesis of non essential amino acids. Concentrations can therefore be reduced when synthetic amino acids are used. The requirements for individual amino acids according to the National Research Council (1984) are indicated in appendix 1.

3.3.2.1. Dietary crude protein concentration and water balance Crude protein concentration of the diet has been suggested to influence water intake, water to feed intake ratios, and the moisture content of excreta and therefore litter moisture (Eley and Hoffman, 1949; Ward *et al,* 1975; Cooke and Raine, 1986; Jordan, 1990; Boden, 1993; Lopez, 1994). However data of other workers suggest this relationship is more equivocal. Malik (1965) could find no effect on excreta moisture when feeding diets containing 140 and 160 g/kg crude protein, and other workers have observed increased water intake as the dietary protein concentration increased, and increased water elimination, but either did not examine (Glista and Scott, 1949; Patrick and Ferrise, 1962; McNab *et al,* 1972; 1973; Ward *et al,* 1975; Marks and Pesti, 1984; Wheelhouse *et al,* 1985) or could detect no consistent relationship with excreta moisture (James and Wheeler, 1949; Wheeler and James, 1950; Patrick, 1955). This was accounted for by a consistent positive relationship between amount of droppings produced, and protein content of the diet, although no real difference in amount of feed consumed between birds fed different protein concentrations occurred. Also in contrast to other work Ogungi *et al* (1983) fed broiler breeder males diets containing 120-180 g/kg crude protein and found that below 160 g/kg there were no effects on water consumption, but decreased excreta moisture.

Although it is generally agreed that increased protein content of diet increases water intake there are differences between source. Much of the work examining water intake and excretion and dietary protein has used incremental concentrations of soyabean meal, often in replacement for maize (Glista and Scott, 1949; James and Wheeler, 1949; Wheeler and James, 1950; Cooke and Raine, 1986). However Wheeler and James (1950) found that, although similar, quantitative effects of soyabean meal were greater than those of fish and meat protein in growing chickens and Patrick (1955) whilst finding soyabean to increase water requirements of broilers found casein to decrease it. In a second study Patrick (1955) tested a number of protein concentrates, including

meat scraps, fish meals, solvent extracted soybean oil meal, solvent extracted cottonseed meal, casein and milk albumin. Again it was concluded that when added in concentrations sufficient to increase protein concentration of a broiler ration from 210 -270 g/kg, they did not always increase water requirements. It appears that there may be an additional relationship between soyabean meal concentration of diets, and the amounts of water consumed and eliminated. Wheeler and James (1950) suggested that the qualitative and quantitative effects of soyabean meal were related to specific diuretic constituents. Soyabean meal contains a high (220 g/kg) concentration of potassium and also a complex carbohydrate which is poorly digested and interferes with water absorption in the distal gut (Pattison, 1989). Therefore its effects on water balance, when used in high concentrations may, reflect the mineral content of the diet as opposed to a protein effect. This effect may cast doubt on work that investigated the response of poultry excreta moisture to dietary protein (Mongin, 1989).

James and Wheeler (1949) pointed out that greater concentrations of water are required for metabolism of protein than for carbohydrate or fats, and so excess protein would require increased water intake. Others have pointed out that proteins and amino acids in excess of needs cannot be stored. Alterations in protein intake affect urinary nitrogen excretion. Results from several studies show that increased dietary protein increases deamination of the excess or unusable amino acids with consequent increases in the excretion of total nitrogen, uric acid and ammonia (Tasaki and Okumura, 1964; Featherstone and Scholz, 1968; Teekel *et al,* 1968; Mc Nab *et al,* 1972, 1973). Uric acid is considered to be the major nitrogenous component of avian excreta (Sturkie, 1965) comprising 60-82 % of nitrogen in urine of the chicken. Increased excretion of uric acid would necessitate increased urine flow and therefore an additional water requirement.

3.3.3. Dietary fat - composition and requirement for fat Fats are the most concentrated energy sources in the diet, and are available from vegetable sources such as maize, soya bean or rapeseed,

or animal sources such as tallow and fish oil. They are also used in diets to increase palatability, reduce dustiness and increase pelleting quality (Scott *et al,* 1982). Fatty acid chain length, extent of unsaturation and nature of esterification all influence intestinal absorbency (Moran, 1989). Unsaturated fats are more easily absorbed and utilised by the bird, therefore if the unsaturation is increased the digestibility is also increased (Freeman, 1976). Linoleic and alpha- linolenic acids are metabolically essential fatty acids and the former the only fatty acid for which the National Research Council (1984) specify a requirement for, stating that the diet of the laying hen should contain 10 g/kg of linoleic acid.

3.3.3.1. Dietary fat quality and water balance There is little information describing the effect of dietary fat quality on water balance. Bray (1985) described a good fat for poultry as containing no more than 19 g/kg unsaponifiable matter and 12 g/kg oxidised fatty acids compared with a poor fat which would contain up to 141 g/kg and 97 g/kg of these constituents respectively. Oxidised fat is poorly utilised by birds and results in high ether extract in the faeces, and a litter which is both low in moisture and greasy. However this did not decrease the moisture content of the excreta, but rather reflected the fact that once litter is capped further droppings were subject to more rapid evaporation which gave a lower moisture content. In contrast, Jordan (1990) stated that poor quality fat, or fat which was poorly digested by poultry would lead to diarrhoea or a soapy scour as well as greasy litter in broilers.

3.3.3.2. Dietary fat concentration and water balance Patrick and Ferrise (1962) fed diets to broilers containing fat at concentrations of 29, 58 and 77 g/kg of diet. All treatments consumed approximately the same volume of water as those which contained no added fat, however no data on water excretion was supplied.

3.3.4. Dietary carbohydrate Carbohydrate is fed to supply energy and consists generally of cereal grains (Scott, 1987). Regardless of grain type, the most important component is starch which comprises 600-700 g/kg of grain weight (Annison, 1990). A number of the polymeric carbohydrate fractions have been suggested to affect water balance of the bird, either directly or indirectly, due to an inability of the bird to produce enzymes capable of hydrolysing them to simple sugars. Remaining simple carbohydrate in the distal part of the digestive tract may influence the ability to absorb water from faeces or urine.

3.3.4.1. Free sugars There are conflicting views as to the effect of free sugars on the water balance of the bird. Neither Rosenburg and Palofax (1956) or Benitez *et al* (1968) could detect an effect of increasing sucrose concentration in the diet on the moisture content of excreta, and Cuervo *et al* (1972 b) found no effect of fructose, glucose or sucrose either individually or in any combination, however Kando and Ross (1962 a) found increased excreta moisture, and a corresponding increase in water consumption *per se.* Jacobs and Scott (1957) showed sucrose addition to water to increase intake of chicks but failed to examine water loss. Carre *et al* (1995) demonstrated water losses in excreta to be greater in chicks fed diets containing 60 g/kg compared to 30 g/kg lactose.

3.3.4.2. Starch - structure and composition Starch represents about 600-700g kg dry matter of most cereals (Annison, 1990), a larger proportion of many roots and tubers, and is a major component of many legumes such as peas and beans (Nigam and Giri, 1961; El faki *et al,* 1984). Structurally it comprises three identifiable alpha linked components, all susceptible to hydrolysis by alpha-amylase which are amylose, amylopectin and an intermediate material. The proportion of each varies from starch to starch. Amylose comprises 15-25% of most starches and is essentially a linear molecule of alpha -1-4 linked D-glucose units, which forms polymers of several

thousand. Amylopectin is the major component of most starches, and consists of a large number of chains, each containing an average of 20-25 alpha-1-4 linked D-glucose residues interlinked by alpha -1-6 glucoside linkages. The physiological effect of dietary starch depends largely on the rate and extent of its digestion and fermentation in the intestine (Jenkins *et al,* 1981; Cummings and Englyst, 1987). A widely held assumption is that starch is completely hydrolysed and absorbed within the small intestine (Dahlqvist and Borgstrom, 1961). It is now widely known from a variety of studies in man (Anderson *et al,* 1981; Sandberg *et al,* 1981; Englyst, 1985; Englyst and Cummings, 1985, 1986, 1987 a and b; Levitt *et al,* 1987; Christl *et al,* 1991) and fowl (Yutste *et al,* 1991) that the extent of starch digestion within the small intestine is variable, and that a substantial amount of starch, depending on physical form, escapes digestion in the small intestine and may enter the colon and be fermented. Starch that resists digestion by pancreatic amylase and becomes available for fermentation in the large intestine has an effect very different from that of starch that is hydrolysed and absorbed in the small intestine. Starch may become resistant to pancreatic enzymes for a number of reasons (Snow and O' Dea, 1981; Englyst and Cummings, 1987 b), both intrinsic and extrinsic. Starch may be physically inaccessible to pancreatic amylase due to the physical form of the food. For example, if starch is contained within whole or partly disrupted plant structure or if rigid cell walls inhibit swelling and dispersion of starch as in parenchyma cells of legumes, or if starch is densely packed (Thorne *et al,* 1983; Wursch *et al,* 1986). Yutste *et al* (1991) iworking with cockerels and chicks suggested physical entrapment of starch to be unimportant but supported a second factor, the nature of starch *per se*. Plant starch is stored in intracellular bodies known as starch granules. Amylose and amylopectin are tightly packed within these granules with a high degree of molecular order, and are associated by hydrogen bonding. Raw granules contain highly crystalline regions.

The shape of granules and the crystal structure within them are characteristic of the plant source and may be one of three types A, B or C as distinguished by their X ray diffraction pattern (Katz,

1937). Cereal starches are usually of the A type. Starches from tubers (e.g. potato) are of B type, and C type are found in legumes and are a combination of A and B types. In general starch granules showing X ray diffraction patterns of B or C types tend to be more resistant to digestion by pancreatic amylase than A types, although degree of resistance is dependant on plant source (Fuwa *et al,* 1980). It seems that different types of starch granules may be degraded by different processes (Leach and Schock, 1961; Mac Gregor, 1983; Steup, 1988). Tuber starches are particularly refractory to digestion by alpha-amylases therefore raw potato starch granules are resistant to degradation by various amylases, including those of the pancreas of chickens (Gallant *et al,* 1972; Coates and Rolls, 1981). Cereal alpha-amylases hydrolyse B granules by surface erosion, whereas the large A granules are degraded from inside to outside following attack at local points. Thirdly, when exposed to heat in the presence of water, during processing, starch becomes gelatinised and dispersed which increases susceptibility of starch to enzyme action (El Faki *et al,* 1984; Holm *et al,* 1985). However as it cools and ages the polymers reform a crystalline structure (retrogradation) which involves formation of interchain hydrogen bonds, and occurs most rapidly for the linear amylose, (retrogradation of amylopectin being limited by its branched structure) characteristically forming a B type pattern. Retrograded starch is resistant to digestion in the small intestine, (Bjork *et al,* 1986; Englyst and Cummings, 1987 b) and the concentration is dependent on processing conditions although it only represents a small fraction of resistant starch. A number of other highly variable extrinsic factors such as amylose-lipid complexes (Larsson and Miezis, 1979; Holm *et al,* 1985), native amylase inhibitors (Shainkin and Birk, 1970; Snow and O'Dea, 1981), transit time of food from mouth to terminal ileum (Chapman *et al,* 1985), concentration of amylase in the gut, amount of starch present, food form (O'Dea and Wong, 1983) and the presence of other food components (Anderson *et al,* 1981) that might retard enzymic hydrolysis as well as antinutritional factors (Jenkins *et al,* 1980; Yoon *et al,* 1983) may also affect digestibility.

Englyst and Cummings (1987 b) have proposed a classification of starch for nutritional purposes in man, based on the intrinsic factors effecting starch digestibility, in which dietary starch is separated into three main groups of different physiological importance on the basis of its physical and chemical properties (Appendix 8.); rapidly digestible starch (RDS), starch likely to be rapidly and completely digested in the small intestine of man; slowly digestible starch (SDS), likely to be digested in the small intestine but at a slower rate and resistant starch (RS), starch likely to resist digestion in the small intestine and become available for fermentation in the large intestine. This later fraction may be further split into physically inaccessible starch (RS1), resistant starch granules (RS2) and retrograded amylose (RS3). It is the RS fraction which is thought to influence reabsorption of water from faeces and urine and therefore effect water balance.

3.3.4.2.1. Dietary starch concentration, composition and water balance Cereal starches are generally more easily digested than root and tuber starches, whilst legume starches have intermediate digestibility (Nitsan and Bartov, 1972; Fleming and Vose, 1979; Coates and Rolls, 1981; Longstaff and McNab, 1987). Englyst *et al* (1992) has shown that compared to foodstuffs such as wheat flour, leguminous foodstuffs are relatively high in resistant starch (Appendix 7.) and Yutste *et al* (1991) showed that starch digestion in young chicks was poorer in foodstuffs that are known to cause problems of high moisture droppings. Dietary ingredients with high concentrations of resistant starch such as tapioca and legumes have been shown to produce wet droppingsin broilers (Cooke and Raine, 1986; Pattison, 1989) although such ingredients also have a high potassium concentration which could explain the observed effects. Recent evidence in man (Cummings *et al*, 1996) casts doubt on the effect of resistant starch on water balance. Resistant starch was found to cause faecal bulking due to increased dry matter excretion, but had no effect on the moisture content of faeces. Mehring *et al* (1962) found that moisture content of excreta was increased when Amijel starch and Peerless pearl starch replaced 500 g/kg of a corn soyabean meal diet but not when dextrinised starch was included in the diet at this high

concentration.

3.3.4.3. Non - starch polysaccharides and their digestive products Cereal grains are important dietary sources of fibre (Wisker *et al,* 1985). Traditional crude fibre and detergent fibre methods for evaluating the fibre fraction of cereals seriously underestimate the fibre content because, although they measure cellulose, they fail to include all the soluble non - starch polysaccharide components. The two major non starch polysaccharide components of cereal fibre which contain highly soluble viscous fractions are the pentosans (arabinoxylans) and the 1-3 1-4 mixed link β-glucans (Henry, 1986). Although similar in structure to cellulose, both being polymers of β-D glucose residues, mixed linked β-glucans differ from the regular β-1-4 structure of cellulose to a mixture of β-D-glucopyranose units, joined by either (1-3) (30 %) or (1-4) (70 %) β-glycosidic bonds (Parrish *et al,* 1960; Perlin and Suzuki, 1962; Luchsinger *et al,* 1965; Clarke and Stone, 1966; Fleming and Manners, 1966; Woodward *et al,* 1983 a and b; Aspinall and Carpenter, 1984). The arabinoxylans comprise β-1-4 linked D-xylose residues of variable length with L-arabinofuranosyl residues, substituted at either the O2 and O3 positions or both positions of the xylose (Neukom, 1976; Bacic and Stone, 1981; Fincher and Stone, 1986; Annison *et al,* 1992) although hexoses and hexuronic acids are also commonly substituted. Arabinoxylans fall into two classes, soluble and insoluble depending on their molecular weight (Montgomery and Smith, 1955), degree of arabinose substitution along the β-1-4-xylan (ratio of xylose to arabinose) (Perlin, 1951; Andrewartha *et al,* 1979), and distribution of side chains (Goldschmid and Perlin, 1963) each of which varies with variety and growing conditions.

Both β-glucans and arabinoxylans are specifically associated with the primary cell walls of starchy endosperm and the thick walled aleurone of grains (rather than maternal tissue where the majority of cellulose is found), where they encrust cellulose fibrils (Fincher, 1975; Neukom, 1976; Forrest and Wainwright, 1977; Fincher and Stone, 1981). It is evident from comparisons

of endosperm cell walls that wheat and rye, together with triticale, differ from barley and oats. The former are characterised by far greater amounts of arabinoxylans, although small quantities of β-glucans and β-glucomannans exist (Podrazky, 1964; Mares and Stone, 1973; Bacic and Stone, 1980). In the latter β-glucans predominate (Aman, 1986; Henry, 1986; Saini and Henry, 1989) comprising some 750 g/kg of the endosperm cell walls (Forrest and Wainwright, 1977). The quantity of β-glucan aswell as the chemical and physical structure in barley are dependant on environmental factors such as climate (dry climates favouring β-glucan deposition), geographical area of production, time and stage of ripening and storage conditions (Laerdal *et al,* 1959; Willingham *et al,* 1960; Gohl and Thomke, 1976; Aastrup, 1979; Hesselman *et al,* 1981, 1983; Woodward and Fincher, 1982; Bourne and Wheeler, 1984; Krogdahl, 1985 b; Henry, 1986) and variety (Mc Clear and Glennie Holmes, 1985; Heen and Varum, 1987, Classen *et al,* 1988, Francesch *et al,* 1994). Consequently the level and degree of detrimental effects varies between batches of barley (Aman, 1986). Dehulling of barley has not reduced and may increase the detrimental effects of β-glucans (Fry *et al,* 1958 a and b; Anderson *et al,* 1961; Classen *et al,* 1985) because of the almost complete endosperm location. The level, chemical and physical structure of pentosans in rye and wheat are also dependant on variety and growing conditions (Medcalf *et al,* 1968; Lineback *et al,* 1977; D' Appolonia and Mac Arthur, 1975; Ciacco and D'Appolonia, 1982; Longstaff and Mc Nab, 1986; Hong *et al,* 1989; Annison, 1990; Izydorczyk *et al,* 1991 a and b) and therefore yearly differences in pentosan content have been recorded (Saastamoinen *et al,* 1989) although Patel and Mc Ginnis (1976) provided conflicting results.

3.3.4.3.1. Cellulose and water balance There is little information concerning the effect of cellulose *per se* on water balance of the bird. Jorgensen *et al* (1996) fed diets containing wheat bran and oat bran to chicks. Both dietary ingredients contain high concentrations of insoluble non - starch polysaccharides (Englyst *et al,* 1989). There was no effect of either on the moisture

content of the excreta although there was a significant increase in excreta output.

3.3.4.3.2. ß glucans and water balance Many workers have made the observation that barley based diets affect the water balance of the bird, causing wet and sticky droppings consistent with the higher β-glucan concentration of barley (Jensen *et al,* 1957; Arscott, 1958; Willingham *et al,* 1959; Rose and Arscott, 1962; Burnett, 1964, 1966; Adams and Naber, 1969; Gohl *et al,* 1977, 1978; Hesselman *et al,* 1981, 1982; Mannion, 1981; De Silva *et al,* 1983; Hesselman, 1983; White *et al,* 1983; Campbell *et al* 1984, 1986; Johnson, 1985; Elwinger and Saterby, 1986; Fincher and Stone, 1986; Herstadt, 1987; Hesselman and Aman, 1986; Rotter *et al,* 1989; Bedford *et al,* 1991; Mc Nab and Smithard, 1992; Francesch *et al,* 1994; Marquardt *et al,* 1994), increase water intake (Arscott, 1958; Berg, 1959; Willingham *et al,* 1959; Arscott and Rose, 1960; Mc Nab and Smithard, 1992), and produce wet litter (Berg, 1959; Herstadt, 1987; Aman, 1986; Krogdahl, 1985 b). The incidence of wet and sticky droppings is lower with advancing maturity of barley at harvest (Thomke, 1972; Hesselman and Thomke, 1982), consistent with a lower β-glucan concentration in late harvested grain. It is likely that other environmental factors effecting the concentration and structure of β-glucan will also effect the incidence of raised excreta moisture. Herstadt (1987) stated that, although many of the negative effects of β-glucans on laying hens were less than in broilers, they still seemed to cause an increase in the amount of sticky droppings.

3.3.4.3.3. Dietary arabinoxylan concentration and water balance A digestion coefficient of 20-30 % has been determined for pentosans (Fraps, 1931; Bolton, 1955) depending on source. Wheat and rye contain high concentrations of pentosan, particularly soluble arabinoxylan. Consequently, numerous workers have observed wet and sticky droppings associated with feeding of wheat (Patel and Mc Ginnis, 1976; Marquardt *et al,* 1994). The effects are particularly apparent in broilers

maintained on low AME (<13 MJ/ME/kg of DM) wheats (Mollah *et al*, 1983; Rogel *et al*, 1987; Annison, 1990) as a result of an increased concentrations of poorly digestible arabinoxylan, and following feeding of isolated water extractable (soluble) and alkali extractable (insoluble) pentosan rich fractions (Choct and Annison, 1992 a and b). A similar response has been observed in birds fed rye as the main cereal source (North, 1933; Halpin *et al*, 1936; Preece and Mc Kenzie, 1952; Fry *et al*, 1958 b; Mac Auliffe and Mc Ginnis, 1971; Fernandez *et al*, 1973 a and b; Patel and Mc Ginnis, 1976; Patel *et al*, 1980; Antoniou *et al*, 1981; Antoniou and Marquardt, 1982; Lee and Campbell, 1983; Campbell *et al*, 1983; Fengler and Marquardt, 1988 a and b; Fengler *et al*, 1988; Pettersson and Aman, 1989; Pawlik *et al*, 1990; Marquardt *et al*, 1994) or as a freeze dried water extract of rye added to a corn or wheat based diet (Fernandez, *et al*, 1973 b; Misir and Marquardt, 1978) or an ethanol precipitate of a sodium hydroxide extract (greatly enriched in soluble viscous pentosans) (Ward and Marquardt, 1987). In contrast to the above mentioned authors, Moran *et al* (1969) found no differences in excreta moisture between broiler chicks fed corn or rye, although Moran *et al* (1970) showed an increase in litter moisture with dietary rye. This anomaly was explained by assuming that accumulated excreta would reduce vaporisation capacity, due to a combination of the hydrophilic nature of the pentosans, and their glueing together of adjacent particles, reducing the exposed surface area upon compaction.

The type, as well as concentration of pentosans in the diet can affect the water content of excreta. Antoniou and Marquardt (1982) fractionated rye pentosans into water soluble and insoluble fractions, and found the latter to produce less watery excreta than birds fed rye, or the water soluble rye pentosan, at identical total pentosan concentration. Those birds fed soluble pentosans produced faeces with the highest water content.

3.3.4.3.4. Pentose sugars Partial depolymerisation of pentosans largely eliminated the anti - nutritive effects of pentosans in broilers, except for watery and sticky droppings (Choct and

Annison, 1992 b). Administration of diets containing pentose sugars (digestion products of pentosan degradation by microbes in the hindgut, and in the small intestine, following treatment of food with xylanases) also gave rise to excreta which was more moist than controls, as also observed by Wagh and Waibel (1966), Schutte (1990) and Schutte *et al* (1992), when feeding D-xylose, or L-arabinose, in graded concentrations in practical type or semi purified diet. Water intake also increased linearly as the dietary concentrations of both pentose sugars increased, but the increase in water intake of chicks fed on the L-arabinose diets were more pronounced than that of chicks fed on the D-xylose diets. Longstaff *et al* (1988) found similar results when offering chicks diets containing D-glucose, D-galactose, D-xylose, L-arabinose, D-galactoronic and glucoronic acid. After only twenty four hours, diets containing either L- arabinose or D-glucoronic acid had much wetter excreta than any of the others. The wet droppings were not linked to excessive drinking at this stage of the experiment, but rather, were suggested to be a direct effect of poor absorption. After fourteen days consumption, wet droppings were observed with all diets, except the D-glucose, and although no direct measurement of water intake was made, water intake appeared to be greater.

3.3.4.3.5. Other non - starch polysaccharide sources Carboxymethyl cellulose (a soluble polysaccharide) when added at 0, 10 and 20 g/kg of diet, increased water intake, and water to feed intake ratio linearly, and the ileal water content was increased from 740 to 860 g/kg by 10 g/kg carboxy methyl cellulose inclusion (Van der Klis *et al*, 1993). Romruen *et al* (1988) with laying hens added graded concentrations (20, 40 and 60 g/kg) of citrus pectin to the diet, and found increased water intake, and an augmentation of the water content of excreta. Burnett (1966) also found that pectins gave very wet and sticky droppings, which adhered to the wire floors of the pens, as did Wagner and Thomas (1977). Recently Jorgensen *et al* (1996) found pea fibre to decrease dry matter content of excreta, and related this to the high pectin content of pea fibre, as shown by Hansen *et al* (1992). Carre *et al* (1995) compared the effects of diets containing 5 g/kg

guar gum and 5 g/kg pectin on excreta moisture of chicks. The water losses in excreta were greater with guar gum than pectin in line with a significantly higher intestinal viscosity with guar gum.

Raised water consumption and excretion, have also been observed in experiments where animals received linseed meal (Kratzer and Williams, 1948; Mac Gregor and Mc Ginnis, 1948), Ground carob (Kratzer and Williams, 1951) and Guar meal (Borcher and Ackerson, 1950; Vohra and Kratzer, 1964), all sources of polysaccharides, and other indigestible hydrocolloids (Nilson and Schaller, 1941; Nilson and Wagner, 1951, 1959). Patterson *et al* (1988, 1989) observed that litter conditions and faecal moisture were noticeably wetter in layers when 910 g/kg wheat middlings were fed, compared to corn soya alfalfa meal fed birds.

3.3.4.3.6. The mechanisms of action of non - starch polysaccharides on water balance and excretion Various explanations have been put forward to explain the effect of non - starch polysaccharides on the water balance of the fowl. Mc Nab and Smithard (1992) have suggested that the low digestibility of β-glucan through an inability of the bird to produce enzymes capable of hydrolysing them to simple sugars, and a propensity to form gels in aqueous media to be responsible for the excretion of wet and sticky droppings, as any remaining simple carbohydrate in the distal part of the digestive tract may influence the ability to reabsorb water from faeces or urine. An increased digesta viscosity may also lead to reduced diffusional rates of digestive enzymes, their substrates and products, which in turn could have a detrimental effect on the rate of enzymic degradation and assimilation of breakdown products, thereby increasing the concentration reaching the hindgut. This could effect water balance directly, or increase bacterial fermentation, which could increase the quantity of osmotically active volatile fatty acids produced (Jorgensen *et al,* 1996). Bedford (1995) concerned with wheat, suggested that its ability to create wet litter was due to the water binding capacity of the arabinoxylans, in particular higher molecular weight arabinoxylans, which have a greater potential to form cross linked hydrogels,

with large water holding capacity than their smaller molecular weight counterparts.

Moran (1982), White *et al* (1983) and Van der Klis *et al,* (1993) have all implicated an increase in the thickness of the unstirred water layer associated with the mucosal surface. This could reduce the rate of water absorption from the gastrointestinal tract, which occurs only after diffusion through the unstirred layer, and consequently the bird would increase water intake to maintain water balance. The latter author noted increased ileal water content, which would support this.

Gohl *et al* (1978) and Fincher and Stone (1986) have suggested that increased microbial populations in the lower gut, from the increase of fermentable carbohydrate with barley based diets, may be responsible for wet and sticky droppings. An increase in viscosity will slow down migration of nutrients, allowing accumulation in the intestine, which may be expected to favour an increased population of micro flora, as observed by (Elwinger and Saterby, 1986), and may increase the proportion of fermentation products, which could effect water reabsorption. Increased solution viscosity as observed with both β-glucan and arabinoxylan is known to increase microbial populations in the small intestine (Feighner and Dashkevicz, 1988). Longstaff *et al* (1988) provided further evidence to suggest that microbial flora may be involved in effecting water balance, when fed non - starch polysaccharides. It was found that caeca from chicks fed on a diet containing 200 g/kg pentoses and uronic acids were heavier, and longer, than those from chicks fed a D-glucose diet. A similar trend was observed when fed 50 g/kg pentoses, uronic acids or L-arabinose due to distension with large quantities of viscous frothy liquid in the process of fermentation. No evidence of fermentation was observed in caeca of chicks fed D-glucose based diets, whereas intermediate levels were found for D-xylose, and galactose, leading to the suggestion that increased fermentation, and microbial proliferation, are observed when high concentrations of indigestible sugars reach the hindgut. Digestibility data suggested that absorption of L-arabinose, and both uronic acids, was poor, even at the very low dietary inclusion

of 50 g/kg whereas absorption of D-xylose was greater than for L-arabinose. Poor absorption is consistent with caecal fermentation, which may be related to the wet excreta, fermentation products interfering with water reabsorption in the caeca, with L-arabinose producing the wettest excreta. Schutte *et al* (1991 and 1992) also fed the breakdown products of arabinoxylans and related the effects on water balance to osmotic properties of unabsorbed pentose sugars and, or, increased volatile fatty acid concentrations in the intestinal tract. Both could result in an inflow of water into the intestinal lumen (Van Weerden, 1959, Hof, 1980), a major function of the caeca being water reabsorption. This theory was supported by Choct and Annison (1992 b) who cited the work of Leegwater *et al* (1974) in rats. The latter fed hydroxy propyl starches, lactose, raw potato starch, polyethylene glycol or magnesium sulphate, and suggested that dietary components which are not completely digested and, or, absorbed in the small intestine, give rise to an increased amount of osmotically active material in the intestinal contents, the amount of which may increase further in the caeca if the non absorbed fragments can be utilised as a substrate by the gut bacteria. This may relate to the inefficient utilisation of five carbon sugars by chicks (Longstaff *et al*, 1988).

Choct and Annison (1992 a) provided further evidence for an effect of microorganisms. They noted the variability in AME values for individual broilers fed wheat diets, to be high compared with that of cereals with lower concentrations of non - starch polysaccharide. This suggested that the antinutritive activity, and wet and sticky dropping problem, were mediated by a highly variable factor (Annison and Johnson, 1989). This factor was suggested to be the gut micro flora because there is considerable variation between birds, in the numbers and types of microorganisms in the digestive tract (Annison, 1989). This was supported by the studies of Wagner and Thomas (1978) and Misir and Marquardt, (1978) who showed that the performance of chicks on cereal based diets were improved with antibiotic supplementation, and that increased ileum anaerobe counts and altered flora occurred in birds fed rye or pectin based diets. Evidence that older birds, which have

more active and stable micro flora, utilise cereal based diets better than young birds (Bolton, 1955; Jeroch, 1987; Johnson, 1987) also supports this observation. Insoluble materials are unlikely to have an effect via microbial fermentation. The connection of the caeca to the intestine is narrow, and has surface cells with long microvilli (Clarke, 1978). It is difficult for insoluble materials to enter the caeca although some soluble polysaccharides may (Van der Klis *et al*, 1993). This may explain the greater effect of isolated soluble than insoluble pentosan fractions on excreta moisture when supplied to wheat based diets.

3.3.5. Miscellaneous dietary factors

3.3.5.1. Feed form There is a considerable divergence of opinion as far as the effects of physical form of the feed on water intake and excretion are concerned. In a comparison of pelleted, and all mash diets, Hoffman and Poitvent (1947) found that feeding of a pelleted mash to broilers reduced the moisture content of litter, however Morris (1947) reported that there was more damp litter where pellets were fed, and Pattee and Rauls (1938), Arscott *et al* (1958) and Arscott and Rose (1960) showed pellet fed chicks to consume more water than those fed mash. Mc Cracken *et al* (1996) also found, in layers, a significant decrease in excreta dry matter for pelleted diets, although the difference was small (347 g/kg for mash based diets vs 329 g/kg for pelleted diets). Grain and mash feeding has also been shown to result in a drier litter condition than all mash feeding in chicks (Hoffman and Tomhave, 1944; Callenbach, 1944) and in layers (Morris, 1947) and in reduced water consumption as a result of mash feeding birds overconsuming in order to wash down particles (Hoffman and Tomhave, 1944). However Eley and Hoffman (1949) found no correlation between feed particle size and moisture content of the droppings, and criticised the work of Hoffman and Tomhave (1944), and Hoffman and Poitvent (1947). It was suggested that the trends these authors observed were the result of variation in protein concentration of the diet, and suggested that, although mash fed birds go to the drinker more often, they actually drink less (smaller drinks). Heat treatment of diets prior to pelleting has been suggested to affect nature of

excreta. However Mc Cracken *et al* (1994) could detect no effect on moisture content of excreta of wheat barley based diets, heat treated to 85 °C for 45 minutes prior to pelleting, or following heat treatment at 80 °C for 10 minutes (Mc Cracken *et al* 1996).

Bailey (1990) suggested that the reports of a positive effect of feed form were probably more a reflection of changes in the quality of food, and the amount eaten, rather than the physical form *per se*. The form of feed influences the cumulative feed consumption of birds, and therefore, considering the relationship between feed and water intake a slight difference in water consumption, and therefore excretion, may be expected.

3.3.5.2. Taste of feed Kare and Pick (1959) conducted an experiment to find out whether the taste of food and water could influence the amount of feed and water intake of chickens. It was noticed that the feed and fluid intake were significantly altered by the use of flavour. Flavour concentration substantially greater than permissible levels were required to elicit these effects.

3.3.5.3. Clays and fillers Latif and Quisenberry (1968) fed diets containing either 25 g/kg or 50 g/kg concentrations of western bentonite and montmorrilimite clays in an attempt to control wet droppings. There was a significant reduction in excreta moisture. Montmorrilimite was more effective than bentonite, and 50 g/kg clay was better than 20 g/kg. Malik (1965) fed sodium bentonite at 20 and 50 g/kg of diet to hens and reported a reduction in excreta moisture. However Sellers *et al* (1980) who fed attapulgite clay at concentrations of 0, 50, 75 and 100 g/kg found that although all attapulgite diets reduced excreta moisture in broilers, in layers neither 50 or 75 g/kg had any effect.

Both Charles and Wildey (1975) and Spandorf *et al* (1975) have fed graded concentrations of kaolin to laying hens and observed a reduction in the moisture content of excreta. The latter

observed a 7 g/kg reduction in moisture content of the excreta for each 10 g/kg kaolin increase.

3.3.5.4. Penicillin Slinger and Pepper (1955) noticed that when chicks and poults were fed a diet to which penicillin had been added they consumed less water even though they showed the expected growth response.

3.3.5.5. Water restriction Intentional water restriction is used in order to prevent raised excreta moisture. It is most commonly used with broiler breeders fed limited quantities of feed, or on a skip a day programme, although occasionally also in laying hens. In the former water restriction occurs on both feed and off feed days. Restriction is done on off feed days since it is assumed that birds will overconsume water on these days due to hunger and boredom. However Bennett and Leeson (1989) indicated that broiler breeders do not consume much water on an off feed day when given free access. They also found that birds drank the same amount of water over a two day feeding period, regardless of whether restricted or not, as when given free access, birds overconsumed on a feed day, but drank little on a non feed day. Therefore in preventing excess water loss, there is a need for water restriction of skip a day fed birds, with particular attention to feed, rather than non feed days.

CHAPTER 4.

PRACTICAL ASPECTS OF DISTURBED WATER BALANCE

4.1. INTRODUCTION

The most common consequence of a water imbalance in poultry i.e. raised excreta moisture poses a number of husbandry problems, as well as more serious economic and environmental problems in modern highly mechanised laying houses. In the broiler industry it is also a major factor in the accumulation of raised litter moisture which in addition to a detrimental effect on husbandry can increase economic loss through increased downgrading of carcasses. In this chapter the main problems in each production system when excreta moisture is increased will be highlighted although emphasis will be placed on the situation in laying houses. This chapter is not intended to be an exhaustive review of all literature relevant to this topic, but is rather aimed at highlighting those works of particular importance.

4.2. PRODUCT QUALITY

4.2.1. Down grading of eggs The proportion of dirty eggs (eggs contaminated by blood, excreta, yolk and dust) produced by laying flocks is a current problem, and a major cause of economic loss in the UK egg industry (Anon, 1993 a). Under EC marketing regulations class A eggs should have a clean shell and not been cleaned, washed and soiled eggs being downgraded (Anon, 1993 b). A recent survey by the U.K. egg inspectorate of 2400 batches of eggs (Anon, 1993 a) indicated that 14 % of all batches had been washed and that 75 % of the washed eggs were from caged systems. Raised moisture content of excreta has been suggested to influence the number of dirty eggs produced in caged layers by adhering to the mesh and marking the eggs as they role away (e.g Halpin *et al,* 1936 a; Walsh, 1993; Herstadt, 1987; Elwinger and Saterby, 1987; Rosenberg, 1955; Kjaer, 1994; Anon, 1993 b). This will lower egg quality and marketability.

However variation in dirty egg numbers from day to day, within a flock is large, and there is little quantitative data to back up these subjective observations.

Appleby and Smith (1991) have monitored weekly dirty egg production from layers in cages. The percentage dirty eggs were generally less than 2 % of all eggs laid, however on two separate occasions over the fifteen week collection period, dirty egg output rose suddenly; 6 % and 18 % dirty eggs being observed in these two week samples. The authors did not explain this variation but it demonstrated that even in good cage design dirty eggs are a highly variable parameter.

4.2.2. Carcass downgrading Raised excreta moisture is one factor, (along with drinker leakage, drinker type, inadequate ventilation and poor insulation), known to cause impaired litter conditions in broiler houses (e.g. Herstadt, 1987; Pattison, 1989; Walsh, 1993). Normal litter should be uniform in consistency and friable. Bray (1985) has shown that it is possible to produce either a wet or a greasy capped litter. The latter involves only surface wetness, that below remaining dry. Areas of wet litter cause birds to avoid sitting on them, and thus the bird's body heat is not available to stimulate litter bacteria action and the situation deteriorates. Martland (1985) showed severe ulceration of skin over the plantar aspect of feet, the caudal aspect of hock joints, and over the sternum in both chicks and turkeys (hock scabs and breast burns) when exposed to wet litter. However Bray (1985) stated that wet litter alone does not necessarily result in hockburn, reporting that increased litter pH and temperature which provide an ideal environment for uric acid splitting bacteria, and therefore ammonia production, which becomes trapped beneath the cap interact to exacerbate the problem. Hockburn can result in economic loss for the producer through downgrading of the carcass, the degree of which is dependant on the size of hock lesions. Small lesions can be removed, but larger lesions may mean loss of premium grade status. Upset of factory routine, an inability to meet orders for premium grade birds and long term effects on customer relations are other problems (Pattison, 1989).

4.2.3. Egg spoilage Rot producing organisms enter the egg through the shell consequently less than 1 % of naturally clean eggs rot during prolonged storage (Brooks and Taylor, 1955). Increased excreta moisture may influence the number of dirty eggs (Rosenburg, 1955). Increased excreta contamination of eggs will increase the percentage of eggs rotting during storage, through an increased initial shell count (Rosser, 1942) and a change in the dominant population of bacteria (Haines, 1939). Gram positive bacteria are numerically dominant on clean or lightly soiled whereas gram negative can be dominant on badly soiled eggs (Board, 1968). Gram negative bacteria are able to penetrate the shell and its membrane most successfully and have properties which favour growth in the contents of the egg, being able to overcome the antibacterial properties of the albumen and having the ability to utilise the protein and protein complexes in the egg fluids as a source of nitrogen for growth (Board, 1968). They are therefore more likely to produce rotten eggs. The commonest contaminants are members of the genera *Alcaligenes, Achromobacter, Pseudomonas, Serratia, Cloaca, Hafnia, Citrobacter, Proteus and Aeromonas.* Increased numbers of eggs lost through rotting can result in economic loss to the producer.

4.3. FLOCK HUSBANDRY

High levels of excreta moisture can reduce the quality of the environment within poultry housing. This can be detrimental to both health and welfare of birds and stockmen. The performance of birds in terms of growth and or egg production and therefore overall efficiency of a unit may also be reduced.

4.3.1. Atmospheric ammonia levels Ammonia originating from nitrogen in excreta is a volatile component, which adversely affects the health of stock and people and reduces productivity of stock occupying the buildings. It is also an environmental nuisance due to its objectionable smell

and its contribution to acid rain (Williams, 1995). It is released during three stages: in the henhouse, during storage, and during application (Van Horne, 1994). The higher the moisture content of excreta, the greater the nitrogen loss via ammonia volatilisation (Voorberg, 1986; Gleadthorpe Contract Report, 1989). Wet conditions and raised relative humidity favour ammonia production, as urate splitting bacteria, which convert uric acid into urea, thrive in excreta of increased moisture content. Ammonia production only occurs with anaerobic decomposition (Esmay and Dixon, 1986). Higher excreta moisture changes the decomposition of excreta from aerobic to anaerobic. Ammonia concentrations are often already high in poultry houses (Kroodsma *et al*, 1988) as priority for environmental control is dependent on temperature, which often results in ventilation rates lower than necessary for maintenance of low concentrations of atmospheric ammonia. Any increase associated with raised excreta moisture may compound the effect, and raise atmospheric ammonia concentrations above those specified in health and safety guidelines. Regulations introduced in Great Britain limit exposure to humans to atmospheric ammonia to a mean concentration of 25 ppm over an 8 hour period, and 35 ppm for 10 minutes exposure (Health and Safety Executive Guidelines, 1986). In stock, guidelines suggest that if birds are exposed to ammonia for an extended period of time concentrations should be below 5 ppm (Ouchi, 1992). Concentrations in excess of those given are associated with a higher rate of bacterial and viral disease in stock (Anderson *et al*, 1964; Donham, 1989; Oyetunde *et al*, 1976; Ouchi, 1992) as a result of compromising the respiratory system, rendering it susceptible to disease challenge. Lowered production performance is also observed, as well as blindness and irritation of mucous membranes. In man, respiratory impairment, and disease of the airways are observed (Donham, 1989) as well as illness in other parts of the body, associated with ammonia levels above 25 ppm. Increased ammonia volatilisation will also have effects following spreading of manure. Ammonia influences the smell of manure, although in terms of odour, loss via volatilisation may only play a small role (Schaefer, 1977).

4.3.2. Humidity Richards (1976) reported that excreta held within a house could account for up to one third of total water production from a flock of laying hens, and Jull (1949) stated that water of vaporisation given off by laying hens, adds considerable moisture to the atmosphere of laying houses, the level of which is influenced by water exhalation, and elimination in droppings. The latter, on quantification, accounted for the larger proportion; up to 75 % of total water loss when moisture content was 850 g/kg. Variation in moisture content of excreta could therefore result in considerable variation in humidity, accentuated if environmental temperature is low and, or, ventilation is insufficient. High relative humidity can cause rapid deterioration of housing structure and electrical equipment, and may also increase the survival of viruses known to cause respiratory disease (Gloster, 1983). Addition of large amounts of water to the house atmosphere requires ventilation and heating, to avoid condensation of moisture on walls and ceilings in cold weather and to facilitate evaporation of moisture. Litter moisture is significantly lower with increased levels of internal air circulation. Increased heat input will increase production costs.

4.3.3. Disease Raised excreta moisture can in broilers, and litter housed layers, predispose, birds to greater coccidiosis infection, and therefore poor performance. Coccidial oocysts mature more rapidly in damp conditions (Jordan, 1990). High moisture excreta is also a more favourable environment for fly larvae development to occur. Increasing fly numbers increases the risk of disease transmission.

4.4. ENVIRONMENTAL ASPECTS

Environmental pollution from agricultural practices is of major international concern. As a result of intensification of animal production systems since the middle of the 1960' s, the quantity and composition of excreta produced exceeds the area of land capable of using it as fertiliser. Consequently, rather than having a beneficial use, manure is now described as a pollutant (Anon,

1990). To control such pollution, legislation is being enacted at the time of writing which both restricts agricultural activity, and penalises farmers for exceeding limits related to waste disposal. Two of the main sources of pollution of agricultural origin are nitrogen, and phosphorus, from excreta. Build up of these elements in the soil, and via leeching, results in pollution of water courses. Such has been the concern related to nitrogen pollution, that a proposed EC directive (COM [88] 708) will limit the number of manure producing animals per hectare of land available for manure spreading to 133 layers, or 285 0-16 wk old birds, or 100 turkeys or ducks, equivalent to a limit of 170 kg^{-1} Ha^{-1} per year total nitrogen, in zones deemed vulnerable with regard to nitrogen leaching. Land available for spreading will be specified, and different limits will be set for zones vulnerable to water pollution from nitrogen compounds. The future code of good agricultural practice for England and Wales will recommend a general limit of nitrogen application of 250 kg^{-1} Ha^{-1} per year to farmers not including nitrogen deposited whilst grazing. Furthermore a closed season for manure application will probably be established, although this will be left to national discretion. Consequently there will be a need to store, (during the closed season, and prior to transport), and transport, large volumes of excreta to allow disposal without contravening regulations or to take to processing plants for conversion to fertiliser for export. A major problem to poultry farms will be handling and disposal of droppings. These become even more difficult to handle when wet (Yoselswitz, 1991). Excreta with a lower moisture content will improve handling, reduce transport costs, and running costs of mechanical drying equipment.

4.5. CONSUMER HEALTH

4.5.1. Eggs Brooks and Taylor (1955) suggested that 90 % of newly layed eggs are free from microorganisms, and suggested the true value may be even higher. However on exposure to the environment, contamination of the egg may occur (Haines, 1939; Stuart and Mc Nally, 1943;

Harry, 1963) mainly as a result of penetration of the shell by bacteria deposited on the surface, post lay. Various workers (Haines, 1938; Rosser, 1942; Forsyth *et al*, 1953; Board *et al*, 1963) have been concerned with the number of microorganisms on the shell, and averages in the range 9.5×10^3 to 3100×10^3 organisms per shell have been reported. Excreta contamination can significantly increase bacterial load as well as numbers of gram negative bacteria which can penetrate the egg shell and contents. Haines (1938) and Board *et al* (1963) suggested that the hen's egg is exposed to external infection from a wide variety of sources, the chief methods being excreta and soil. Raised excreta moisture can increase the proportion of eggs contaminated with excreta. Contamination of the egg shell by excreta increases risk of contamination of internal contents by salmonella, and other bacterial organisms (Humphrey *et al*, 1989). Other workers have shown a relationship between dirty eggs and increased microbial contamination (Johns & Berard, 1946; Forsyth *et al*, 1953). There are potential dangers to human health of using eggs contaminated with excreta (Anon, 1988).There are also risks of cross contamination to clean eggs either directly, or through handling.

Salmonella represents one of the most important health risks from egg consumption. Any faecal material adhering to shells, cage floors, feather, feet contaminated with salmonella could put eggs at risk from contamination. The most widely accepted route of contamination into the egg by salmonella is through penetration of the organisms from the shell surface (North, 1989) although transovarian transmission is also reported for *S. enteriritidis*. Salmonella organisms are present in many areas of the environment, and are persistent. Contamination of the shell may occur during lay, or through microscopic shell cracks, or as a result of bacteria passing through the natural pores of the shell when eggs are kept at high temperatures / humidity (World Health Organisation, 1988). Such penetration is however not readily achieved as the egg presents a complex series of defences (Tranter & Board, 1982). Consequently reported numbers of bacteria in eggs are low.

Such levels according to a government publication are unlikely to be harmful to most healthy adults. However as a precaution the egg laying industry is required under EC rules to remove from the shell egg market cracked or dirty eggs.

4.5.2. Carcasses Raised excreta moisture can cause consumer health problems in broilers. Bird feathers become dirty as excreta moisture increases. If this happens near slaughter time the birds will be dirty and possibly microbially contaminated when slaughtered. This may increase overall contamination in the plant.

4.6. CONCLUSION

In conclusion the preceding review of current literature shows that:

(i) Diet is a major factor affecting the water balance of birds. Those dietary factors of particular importance are in descending order of importance:

a) The major minerals (sodium, potassium, phosphorus, calcium and magnesium)

b) Protein concentration and quality

c) Resistant starch and both soluble and insoluble non - starch polysaccharide

(ii) There are many non - nutritional factors affecting water balance. Therefore to quantify the effects of diet on water balance there is a need to standardize environmental temperature and humidity and all experimental data should be collected under the same management system using the same drinker type, cage type and with birds of the same breed, age and rate of lay.

CHAPTER 5.

THE EFFECT OF DIETARY CONCENTRATIONS OF SODIUM, PHOSPHORUS, POTASSIUM AND CALCIUM ON EXCRETA MOISTURE AND WATER INTAKES OF LAYING HENS

5.1. INTRODUCTION

Dietary mineral composition can have a major effect on the water intake and excretion of poultry. Increased osmolarity of extracellular fluid provokes thirst in the fowl, following dehydration of osmoreceptors in the hypothalamus (Stallone and Braun, 1986). Increased water intake stimulates the kidneys to make appropriate changes in both mineral and water excretion, in order to maintain fluid and electrolyte balance (Hill *et al,* 1979). The volume of urine excreted is dependent on the amount of solute for excretion, because the concentrating capacity of the kidneys is limited (Lote, 1982) and the fractional water reabsorption across the epithelium of the copradeum and large intestine is dependant on the level of salt loading, and level of urine flow (Skadhauge, 1968).

Sodium and potassium are the principal osmotically active electrolytes in extracellular, and intracellular, fluids respectively. High dietary intake of either mineral would give a large osmotic change. High potassium intakes have been shown to increase the amount of potassium filtered at the kidney glomerulus, and increase the renal tubular secretion of potassium (Mason and Scott, 1972; Beal *et al,* 1973). Excess phosphorus that is absorbed from the gut has been shown to be excreted in urine, (Fussell, 1960), and the urinary excretion was equivalent to the amount absorbed from the gastrointestinal tract. Foorman and Leeson (1959) suggested that excess calcium may lead to the stimulation of thirst centres in the mammalian brain, resulting in polydipsia and polyuria. However any such relationship in avian species is unknown. There is high turnover and precise hormonal control of calcium metabolism in the laying bird.

Many of the workers whose interest has been in the relationship between the quantity of water drunk, and that lost in excretion, relied on making daily collections of excreta, and weighing and drying them to constant weight. Since the rate of evaporation from freshly expelled excreta is

high, estimates of the water content of droppings collected at intervals from open trays were inaccurate (Hill *et al*, 1979).

There is little information that quantitatively describes the increase in excreta moisture to different levels of dietary minerals. Four separate experiments were carried out in this project to measure, quantitatively, the response in water intake, and excreta moisture, of laying hens, to dietary concentrations of sodium, calcium, potassium and phosphorus, that met or exceeded the birds requirements. A second objective of the first experiment was to determine, quantitatively, the water evaporation during excreta collection in open trays, by a comparison with excreta collection under oil (no evaporation) to produce a correction factor to overcome the inaccuracies in open tray collection. A fifth experiment examined whether excreta moisture differed when two different sodium salts (bicarbonate or chloride) were fed, and if the effects of dietary sodium and phosphorus excess were additive.

5.2. MATERIALS AND METHODS

5.2.1. Experiment 1 The first objective of this experiment was to measure, quantitatively, the effect of varying dietary sodium concentrations on the excreta moisture of laying hens. However a second objective was to determine, quantitatively, the water evaporation during excreta collection in open trays, by a comparison with excreta collection under oil (no evaporation).

Forty eight, 38-week-old ISA Brown laying hens were caged in individual wire floored cages (50 x 45 x 45 cm), arranged in four tiers, within an environmentally controlled room. Each cage had an individual feed hopper, water trough and excreta collection tray. The birds were maintained under a 14L : 10D lighting regime at 24± 1 °C, and 80± 5% relative humidity.

Diets were practical laying hen rations (11.69 MJ/kg of ME and 168 g/kg of crude protein) that were identical in nutrient composition, except in sodium concentration (Table 5.1.). The diets were formulated to contain six levels of sodium, (1.6, 5.5, 9.4, 13.3, 17.2 and 21.1 g/kg) from sodium chloride. Sodium chloride replaced washed sand in the diet. Each bird had *ad libitum* access to one of the six diets and water throughout the 16 d feeding period.

Two 48 h excreta collections were carried out on days 7 and 8 and on days 15 and 16. Each 48 h collection period consisted of two separate 24 h collections, once on open trays placed under the cage, and once on trays that contained a 5 cm depth of mineral oil. The mineral oil prevented moisture loss from the excreta during the 24 h collection period. The moisture contents of the excreta were determined by drying at 60 °C in a forced air oven for that collected on open trays or by drying at 105 °C in a forced air oven for that collected under oil. Water and feed intakes were determined for each 24 h collection period. Both were determined by weighing the appropriate trough at the beggining and the end of the collection period. Water intakes were adjusted for loss

of water vapour from the drinkers by correcting for the amount of water lost from an identical drinker on each tier in a position inaccessible to the laying hens. Only on egg laying days were data used in calculation of treatment means to reduce variation as a result of a response in water balance to egg formation, (Wood - Gush and Horne, 1970).

The experiment was designed as a randomised block analysis of variance, with blocking factors of cage tier level and collection day. Treatment sums of squares were partitioned into a set of orthogonal linear and quadratic polynomial regression components, using the GENSTAT statistical package (Lawes Agricultural Trust, 1984). Regression coefficients were obtained by fitting data to a simple linear model. Multiple regression analyses, with water intake, and feed intake, as independent variables and excreta moisture as a dependent variable, were also carried out.

5.2.2. Experiment 2 The objective of this experiment was to measure quantitatively the effect of six levels of dietary phosphorus on excreta moisture, and water intake, of laying hens.

Forty eight, 42-week-old ISA Brown laying hens were housed in the same cages and given identical lighting, temperature and relative humidity, as described in experiment 1. The six diets were typical for laying hens (11.69 MJ/kg of ME and 168 g/kg of crude protein) and had identical nutrient compositions except in their phosphorus concentration (Table 5.1.). The feeds provided six levels (3.0, 4.0, 5.0, 7.5, 10.0 and 20.0 g/kg) of available inorganic phosphorus as dicalcium phosphate. Birds had *ad libitum* access to both feed and water, throughout the 8 d feeding period.

Water intakes and feed intakes were measured, and all excreta were collected, on the final two days of the feeding period. Excreta was collected in open trays, and excreta moisture levels determined by drying total excreta at 60 °C. Data were then corrected for loss of moisture to the environment,

Table 5.1. Composition and analysis (g/kg) of basal diets[1]

	Experiment No[2]				
	1 (Na)	2 (P)	3 (Ca)	4 (K)	5 (Na/P)
Wheat[3]	447.00	447.00	447.00	335.80	432.00
Barley	189.00	189.00	189.00	.....	183.00
Sunflower meal	.....	.....	.....	20.00	.....
Maize flour	.....	.....	.....	180.00	.....
Maize gluten meal	79.80	79.80	79.80	.....	79.80
Dehulled soya bean meal	36.10	36.10	36.10	.....	36.10
Fish meal	21.50	21.50	21.50	.....	21.50
Meat and Bone meal	34.40	30.00	34..40	.....	34.40
Lysine Hydrochloride	4.30	4.30	4.30	11.10	4.30
Methionine	1.50	1.40	1.40	1.40	1.40
Tryptophan	.....	.....	.....	1.00	.....
Soya oil	43.50	53.50	43.50	55.00	47.50
Limestone	80.00	77.30	52.70	83.50	.....
Dicalcium phosphate	.....	.....	.....	16.00	.....
Sodium chloride	.....	.....	.....	0.40	.....
Potassium chloride	.....	.....	.....	.....	.....
Magnesium chloride	.....	.....	.....	.....	.....
Sand[4]	50.00	47.10	77.30	100.00	.....
Vitamin mineral premix[5]	12.50	12.50	12.50	12.50	12.50
Analysis (calculated)					
Potassium	4.30	4.30	4.30	2.30	4.30
Sodium	1.60	1.50	1.60	1.50	2.50
Calcium	40.60	39.00	30.00	40.50	40.10
Phosphorus	4.10	2.90	4.10	4.20	4.10
Crude protein	168.60	166.50	168.60	164.90	168.00
Lysine	0.89	0.88	0.89	0.88	0.89
Methionine	0.48	0.48	0.48	0.48	0.48
ME (MJ of ME/kg)	11.69	11.69	11.69	11.69	11.69

1. All diets in each experiment were identical to the respective basal diet except for in the mineral in question which increased in concentration at expense of washed sand as described in text
2. Diet numbers 1-5 refer to trials 1-5 respectivly
3. All componants are expressed in g/kg of feed
4. Washed builders sand use as a filler in formulating experimental diets
5. Comprised ash (890 g/kg), calcium (250 g/kg), methionine (80 g/kg), sodium (88.0 g/kg), copper (cupric sulphate 400 mg/kg), vitamin A (480000 i.u./kg), vitamin E (alpha tocopherol acetate 480 i.u./kg), vitamin D3 (240000 i.u./kg)

using the correction factor obtained in experiment 1. Water and feed intakes were determined by weighing the appropriate trough at the beggining and the end of the collection period. Water intakes were adjusted for loss of water vapour from the drinkers by correcting for the amount of water lost from an identical drinker on each tier in a position inaccessible to the laying hens. Data were used to calculate treatment means only if an egg had been laid that day. Statistical design and analyses were carried out as in experiment 1, except that cage tier level was the only blocking factor.

5.2.3. Experiment 3 The objective of this experiment was to measure quantitatively the change in excreta moisture, and water intake, of laying hens when fed six levels of dietary calcium.

The experimental protocol was identical to experiment 2 except in the age of the laying hens (44 week old) and in the composition of the experimental diets (Table 5.1.). Different levels of calcium carbonate, in replacement for washed sand, provided six levels (30.0, 35.0, 39.0, 41.0, 45.0 and 50.0 g/kg) of dietary calcium.

5.2.4. Experiment 4 The objective of this experiment was to investigate quantitatively the effect on excreta moisture, and water intake, of dietary potassium level in laying hens.

All aspects of experiment 4 were identical to experiments 2 and 3, except in the age of the laying hens (47 weeks old) and the composition of the experimental diets. The diets provided six levels of potassium (2.3, 5.0, 7.5, 10.0, 15.0 and 20.0 g/kg) supplied as potassium carbonate (Table 5.1.), in replacement for washed sand. A proportion of wheat was replaced with maize starch in the formulation of the diets used in this experiment, in order that the potassium content of the basal diet could be minimised.

5.2.5. Experiment 5 The first objective of this experiment was to measure the changes in excreta moisture, when two different levels of two sodium salts, (sodium bicarbonate and sodium chloride), were included in the rations of laying hens (46 weeks old). The second objective was to measure the increase in excreta moisture, when two levels of phosphorus were fed to laying hens, and the third was, to examine whether there were any sodium level x sodium salt x phosphorus level interactions. A wholly randomised block analysis of variance was used arranged as a 2 x 2 x 2 factorial.

The cage dimensions and the environmental conditions were the same as used in experiments 2, 3 and 4. Eight diets were compared (Table 5.1.) in which one of two levels of sodium (2.5 g/kg or 12.5 g/kg) provided as either sodium bicarbonate or sodium chloride, and one of two levels of available inorganic phosphorus (4.0 g/kg or 20.0 g/kg) as dicalcium phosphate, were fed in replacement for washed sand (Table 5.2.).

5.2.6. Feed analysis Samples of feeds for each experiment were ground to pass a 0.5 mm mesh screen, and were subsequently subjected to standard proximate analysis (A.O.A.C., 1990). Sodium, calcium and potassium concentrations were determined with an atomic absorption spectrophotometer (Smith-Hieftje 1000, Thermo Jarrell Ash Corporation), following wet digestion (A.O.A.C, 1990). Inorganic phosphorus was determined using a colorimetric technique (MAFF, 1986) in which concentration of phosphorus in a trichloroacetic acid extract is determined spectrophotometrically as the yellow phospho- vanado- molybdate complex, at 400 nM. Diets which failed to conform to the calculated analysis +/- 10% (Table 5.1) were re- mixed and re-analysed.

Table 5.2. Composition of premixes for experiment 5

Diet	Sodium chloride	Sodium bicarbonate	Dicalcium phosphate	Calcium carbonate	Sand
Sodium bicarbonate (12.5g/kg)/ Phosphorus (20.0g/kg)	0.00	46.90	82.50	19.10	0.00
Sodium chloride (12.5g/kg)/Phosphorus (20.0g/kg)	28.00	0.00	82.50	19.10	12.35
Sodium bicarbonate (12.5g/kg)/ Phosphorus (4.0g/kg)	0.00	40.40	5.00	76.00	8.76
Sodium chloride (12.5g/kg)/ Phosphorus(4.0g/kg)	28.00	0.00	5.00	76.00	33.00
Sodium bicarbonate (2.5g/kg)/ Phosphorus (20.0g/kg)	0.00	3.60	82.50	19.10	39.70
Sodium chloride (2.5g/kg)/ Phosphorus (20.0g/kg)	2.50	0.00	82.50	19.10	37.80
Sodium bicarbonate (2.5g/kg)/ Phosphorus (4.0g/kg)	0.00	3.60	5.00	75.00	58.30
Sodium chloride (2.5g/kg)/ Phosphorus (4.0g/kg)	2.50	0.00	5.00	77.00	59.20

5.3. RESULTS

5.3.1. Experiment 1 (see Table 5.3.) There was a linear decrease ($p<0.001$) in feed intake, with increased dietary sodium concentrations. However, increased dietary sodium concentrations still gave linear increases ($p<0.001$) in daily sodium intakes, water intakes ($p<0.001$)(Figure 5.1.) and the ratios of water to feed intake ($p<0.001$). There were therefore linear increases ($p<0.001$) in the weights of water excreted, the moisture contents of the excreta (g/kg) (Figure 5.1.), and total outputs (fresh weight) of excreta.

The moisture content of the excreta (g/kg) was consistently higher when it was collected under oil, compared to collection on open trays. The difference in the two measurements was a constant 30.8±10.3 g/kg at all moisture levels. There was no evidence ($p>0.05$) that excreta with higher moisture contents lost more water during open tray collection. The difference obtained was used in all subsequent experiments, to correct all data where excreta were collected on open trays.

5.3.2. Experiment 2 (see Table 5.4.) There was no effect of dietary phosphorus concentration on feed intake ($p>0.05$) so increased dietary phosphorus concentration produced a linear increase ($p<0.001$) in phosphorus intake. Increased dietary phosphorus concentration increased water intakes ($p<0.001$) (Figure 5.2.) and water to feed intake ratios ($p<0.001$). There were also linear increases ($p<0.001$) in the weight of water excreted, the moisture contents of the excreta (g/kg) (Figure 5.2.) and total outputs (fresh weight) of excreta.

5.3.3. Experiment 3 (see Table 5.5.) Despite increased daily calcium intakes ($p<0.05$), increasing levels of dietary calcium between 30.0g/kg and 50.0g/kg had no effect ($p>0.05$) on the moisture contents of excreta, or the water or feed intakes of the laying hens.

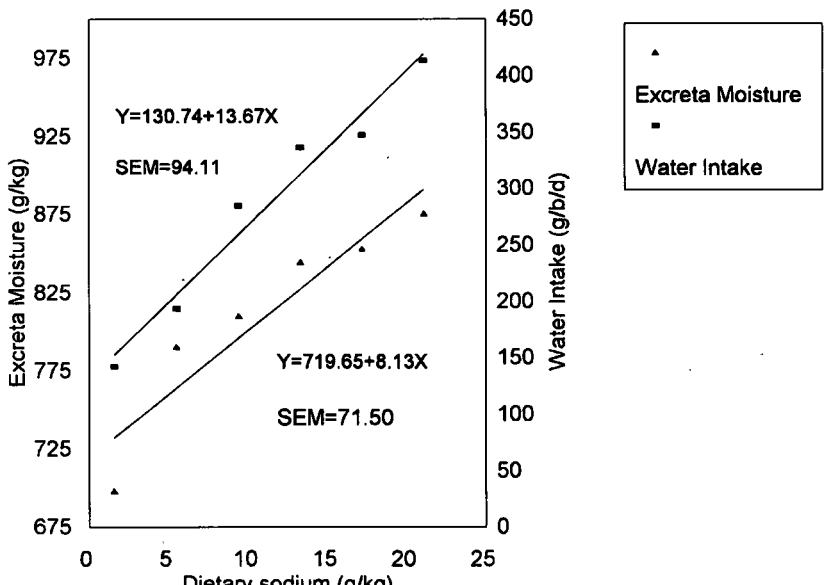


Figure 5.1. The effect of dietary sodium concentration on the excreta moisture and water intake of laying hens

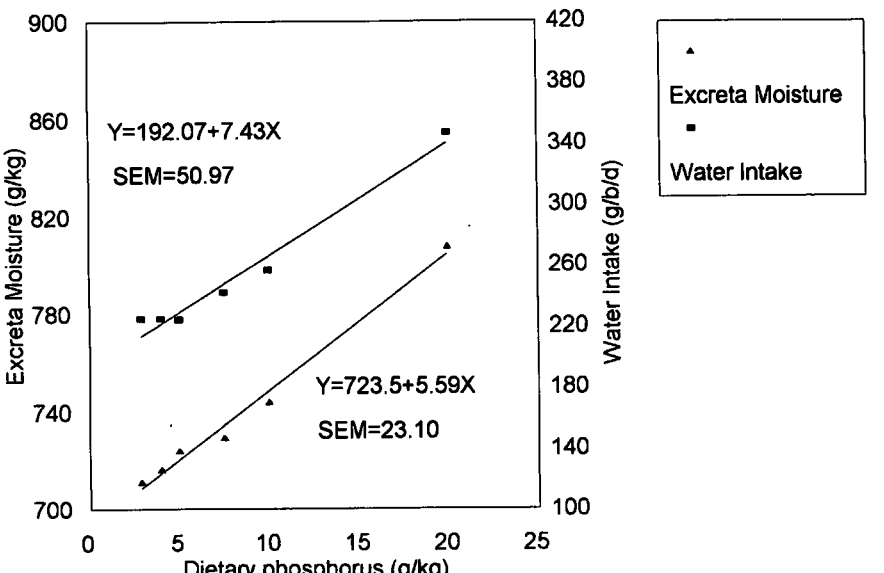


Figure 5.2. The effect of dietary phosphorus concentration on the excreta moisture and water intake of laying hens

Table 5.3. The effect of dietary excess of sodium on excreta moisture, water intake and other parameters of laying hens

	Dietary sodium (g/kg)							
	1.60	5.50	9.40	13.30	17.20	21.10	SEM[1]	Significance[2]
Excreta moisture (oil) (g/kg)	697.7	790.1	810.0	844.4	853.0	875.3	71.50	***
Excreta moisture (free) (g/kg)	650.5	739.6	786.1	809.4	848.3	852.2	71.50	***
Water intake (g/b/day)	141.9	193.5	284.3	336.2	347.0	412.7	94.11	***
Food intake (g/b/day)	112.8	99.8	92.9	73.8	60.1	48.1	29.95	***
Water : feed ratio (g/g)	1.43	2.19	3.21	4.94	7.14	9.64	3.36	***
Sodium intake (g/b/day)	0.18	0.55	0.88	0.98	1.03	1.01	0.33	***
Total excreta (g/b/day)	72.3	99.6	132.7	141.1	148.4	176.0	32.76	***
Water excreted (g/b/day)	49.0	76.1	105.6	117.6	126.3	149.9	48.90	**
Dry matter excreted (g/b/day)	23.2	23.4	27.1	23.5	22.0	26.0	12.76	NS

1. df=39 (all parameters)
2. Significance level of the slope (b) where y=a+bx *** ($p<0.001$), ** ($p<0.01$), * ($p<0.05$), NS ($p>0.05$)

Table 5.4. The effect of dietary excess of phosphorus on excreta moisture, water intake and other parameters of laying hens

	Dietary phosphorus (g/kg)							
	2.90	4.00	5.00	7.50	10.00	20.00	SEM[1]	Significance[2]
Excreta moisture (g/kg)	711.3	716.4	724.1	729.5	744.0	807.5	23.10	***
Water intake (g/b/day)	224.8	225.1	224.2	242.2	256.6	346.7	50.90	***
Food intake (g/b/day)	135.2	141.8	148.0	151.5	147.3	146.8	22.80	NS
Water : feed ratio (g/g)	1.73	1.60	1.52	1.66	1.77	2.41	0.410	**
Phosphorus intake (g/b/day)	0.39	0.57	0.74	1.13	1.41	2.93	0.270	***
Total excreta (g/b/day)	105.8	115.8	116.7	117.2	122.9	182.9	26.80	**
Water excreted (g/b/day)	75.3	83.0	84.5	85.3	91.5	148.7	23.40	**
Dry matter excreted (g/b/day)	30.5	32.7	32.2	31.8	31.3	34.1	4.90	NS

1. df=39 (all parameters)
2. Significance level of the slope (b) where y=a+bx *** ($p<0.001$), ** ($p<0.01$), * ($p<0.05$), NS ($p>0.05$)

Table 5.5. The effect of dietary excess of calcium on excreta moisture, water intake and other parameters of laying hens

	Dietary calcium (g/kg)							
	30.00	35.00	39.00	41.00	45.00	50.00	SEM[1]	Significance[2]
Excreta moisture (g/kg)	718.00	729.00	677.00	713.00	695.00	700.00	42.90	NS
Water intake (g/b/day)	232.10	185.80	192.30	191.50	179.80	174.80	53.79	NS
Food intake (g/b/day)	144.40	132.80	137.10	131.70	120.80	120.70	31.15	NS
Water : Feed ratio (g/g)	1.59	1.40	1.43	1.65	1.70	1.48	0.54	NS
Calcium intake (g/b/day)	4.33	4.64	5.35	4.99	5.44	6.03	1.33	*
Total excreta (g/b/day)	109.40	105.20	101.50	99.30	77.90	93.20	25.78	NS
Water excreted (g/b/day)	79.00	76.70	68.50	71.40	54.60	65.50	19.20	NS
Dry matter excreted (g/b/day)	30.40	28.60	33.00	27.90	23.30	27.70	7.97	NS

1. df=39 (all parameters)
2. Significance level of the slope (b) where y=a+bx *** ($p<0.001$), ** ($p<0.01$), * ($p<0.05$), NS ($p>0.05$)

5.3.4. Experiment 4 (see Table 5.6.) There was a linear ($p<0.001$) decrease in feed intake with increasing dietary potassium concentration. However increased dietary potassium concentrations gave linear increases ($p<0.001$) in daily potassium intakes, water intakes (Figure 5.3.) and the ratio of water to feed intakes ($p<0.001$). There were linear increases ($p<0.001$) in the weights of water excreted, the moisture contents of the excreta (g/kg) (Figure 5.3.), and total outputs (fresh weight) of excreta.

5.3.5. Experiment 5 (see Table 5.7.) Increased dietary phosphorus and sodium both raised ($p<0.001$) excreta moisture, water intakes and total excreta outputs. There were no sodium salt x sodium level interactions ($p>0.05$) for water intakes, water to feed intake ratios, or the moisture content of excreta. There was a sodium level x phosphorus level interaction ($p<0.05$) for excreta moisture, but no interactions for water intakes, or water to feed intake ratios ($p>0.05$).

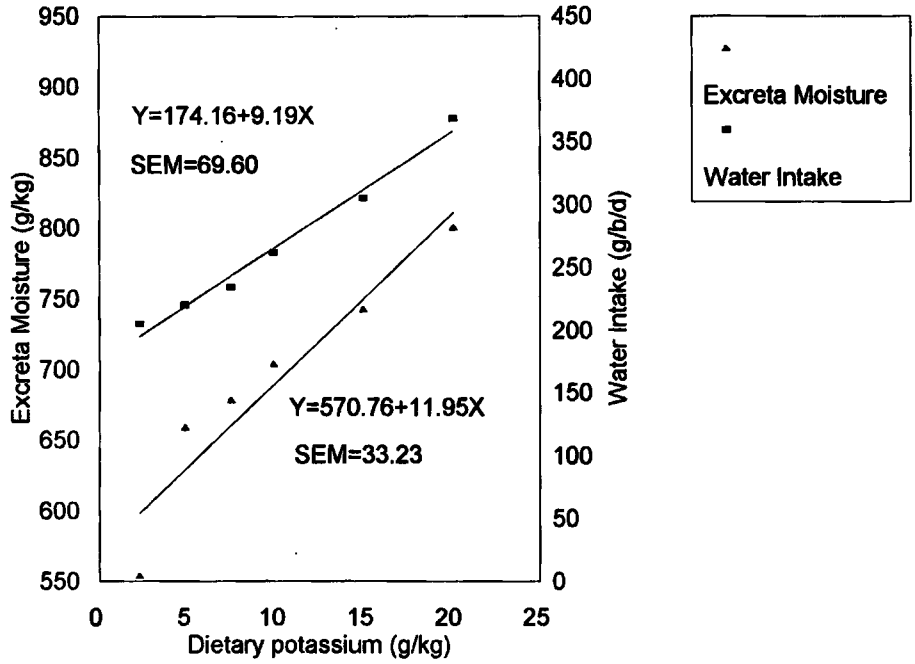


Figure 5.3. The effect of dietary potassium concentration on the excreta moisture and water intake of laying hens

Table 5.6. The effect of dietary excess of potassium on excreta moisture, water intake and other parameters of laying hens

	Dietary potassium (g/kg)							
	2.30	4.90	7.50	9.90	15.00	20.00	SEM[1]	Significance[2]
Excreta moisture (g/kg)	553.9	659.1	678.3	703.7	742.8	800.4	33.20	***
Water intake (g/b/day)	205.0	220.0	234.0	262.0	305.0	368.0	69.60	***
Food intake (g/b/day)	119.5	113.1	97.4	97.6	87.1	68.9	23.50	***
Water : Feed ratio (g/g)	1.75	1.96	2.5	2.72	3.60	6.12	1.36	***
Potassium intake (g/b/day)	0.27	0.55	0.73	0.96	1.30	1.38	0.26	***
Total excreta (g/b/day)	60.9	72.0	77.7	81.0	86.4	89.9	21.70	***
Water excreted (g/b/day)	31.9	45.3	50.4	54.7	61.5	68.6	15.36	***
Dry matter excreted (g/b/day)	29.0	26.7	27.2	26.3	24.9	20.4	7.30	*

1. df=39 (all parameters)
2. Significance level of the slope (b) where y=a+bx *** ($p<0.001$), ** ($p<0.01$), * ($p<0.05$), NS ($p>0.05$)

Table 5.7. The interaction of sodium, phosphorus and sodium source on excreta moisture, water intake and other parameters of laying hens

	Dietary sodium/ phosphorus (g/kg)								
	Bicarbonate				Chloride				
Sodium	12.5	12.5	2.5	2.5	12.5	12.5	2.5	2.5	SEM[1]
Phosphorus	20.0	4.0	20.0	4.0	20.0	4.0	20.0	4.0	
Excreta moisture (g/kg)	896.6	859.1	800.5	724.9	893.5	864.6	806.0	740.30	24.70
Water intake (g/b/day)	440.7	402.2	268.5	202.0	482.4	415.1	290.0	235.8	36.47
Food intake (g/b/day)	109.3	110.4	131.2	144.0	117.7	118.7	146.8	158.3	27.04
Water : food ratio (g/g)	4.05	3.70	1.82	1.45	4.14	3.53	1.96	1.49	0.32
Phosphorus intake (g/b/day)	2.46	0.44	2.62	0.58	2.35	0.47	2.93	0.22	0.46
Sodium intake (g/b/day)	1.54	1.38	0.33	0.36	1.47	1.48	0.36	0.39	0.22
Total excreta (g/b/day)	267.0	206.8	173.0	129.7	299.8	259.6	174.0	134.2	28.96
Water excreted (g/b/day)	186.0	149.9	138.5	93.0	267.9	225.7	139.6	99.9	34.75
Dry matter excreted (g/b/day)	30.9	33.9	31.4	36.7	31.9	33.9	34.5	34.9	5.16

1. df=40 (all parameters)

5.4. DISCUSSION

5.4.1. Comparison of minerals The quantitative estimates from these experiments for increases in excreta moisture and water intake, due to dietary sodium, were similar to other published estimates (Table 5.8.). In contrast the estimates derived for potassium were much greater than described by Kando and Ross (1962 a & b), who used molasses to alter potassium levels in the diet. The source of dietary potassium could effect determined values. Inorganic potassium salts have been shown to produce a greater response than potassium supplied as molasses on excreta moisture and water intake (Kando and Ross, 1962 a & b; Hijikuro, 1976). However this was not enough to explain the differences between the current estimates and those previously published.

Current estimates of excreta moisture with increased dietary concentrations of sodium and potassium conflict with previously published data (Table 5.8.), which suggest that dietary sodium will increase excreta moisture to a greater extent than dietary potassium. When related to dietary intake rather than dietary concentration the effect of sodium was greater in the current experiment. This difference could be explained by the large depression in feed intake at high dietary sodium concentrations.

No previous estimates for the effect of dietary phosphorus on moisture content of excreta, or water intake, were found. The responses in excreta moisture and water intake to increasing dietary phosphorus were less than for potassium and sodium (Table 5.8.). This apparent lower sensitivity may reflect the greater requirement of the bird for phosphorus than sodium or potassium. Additionally, feed intake, and therefore dry matter content of faeces, was unaffected by dietary phosphorus, unlike dietary sodium and potassium.

Variation in dietary calcium between 30 g/kg and 50 g/kg had no effect ($p>0.05$) on excreta

Table 5.8. A comparison of experimentally and literature derived linear estimates for excreta moisture and water intake in diets varying in concentration of one mineral

Mineral		Estimates from present experiments	Estimates derived from other published evidence
Sodium	Excreta moisture (g/kg)	Y=693.7+9.04±1.57[1]x	Y=726.3+8.80x[2]
	Water intake (g/b/d)	Y=130.7+13.67±1.32x	Y=113.0+14.0x[3] Y=246.9+16.0x[4]
Potassium	Excreta moisture (g/kg)	Y=570.7+11.95±2.02x	Y=835.8+3.30x[2]
	Water intake (g/b/d)	Y=174.2+9.195±0.62x	Y=37.6+2.56x[5]
Phosphorus	Excreta moisture (g/kg)	Y=723.5+5.59±0.31x	.....
	Water intake (g/b/d)	Y=192.1+7.43±0.64x	.....
Calcium	Excreta moisture (g/kg)	Y=755.47+(-1.24±1.15x)	.....
	Water intake (g/b/d)	Y=204.19+(-0.41±0.80x)	.....

1. Standard error of coefficient
2. Kando and Ross (1962 a) in day old chicks
3. Hijikuro (1976) in growing pullets
4. Damron and Kelly (1987) in laying hens
5. Kando and Ross (1962 b) in day old chicks

moisture or water intake, in line with data of Roland and Caldwell (1985), who found that only extremely low levels of dietary calcium affected excreta moisture of laying hens.

A failure of differing workers to standardise environmental and collection conditions, during dry matter determination of excreta, makes comparison of the literature difficult and imprecise (Hill *et al,* 1979). The standardisation of collection method, and correction for loss of moisture to the environment, as carried out in the present experiments, allows this problem to be overcome. Consequently it can be concluded that when in dietary excess as the inorganic salt, the order of importance per unit weight in determining excreta moisture in the laying hen is potassium> sodium> phosphorus. An increase in potassium or sodium gave approximately twice the response of an equivalent increase in dietary phosphorus. Potassium and sodium are therefore the two minerals of particular practical importance.

Sodium concentration of plant material is low, therefore dietary sodium is generally provided in diets as the free chloride salt. As a comparatively low priced and non toxic additive it is subject to variation, due to formulation mistakes, as well as separation from the bulk of the diet during storage. Potassium is generally present in proprietary rations, far in excess of the nutrient requirement of 1.5 g/kg (National Research Council, 1984), and is subject to considerable variation as potassium concentration in plant material is high. By controlling the quantity of dietary components rich in potassium, used in ration formulation, and supplementary sodium levels a considerable proportion of variation in excreta moisture could be controlled.

5.4.2. Sodium phosphorus interactions Experiment 5 indicated that the effects of dietary sodium and phosphorus level on water intakes and excreta moistures of hens were additive. There was a significant ($p<0.05$) phosphorus level x sodium level interaction for excreta moisture. However the sodium x phosphorus interaction sum of squares for excreta moisture was small, relative to

those of the main treatment sums of squares. The interaction occurred in diets containing high levels of sodium and phosphorus, and therefore may be a result of a limitation on water excretion at very high levels. Although Shoemaker (1972) stated that as little as 6% of filtered water may be reabsorbed in the fowl kidney, both Kando and Ross (1962 b), and Hijikuro (1976), observed a point where moisture content of excreta no longer increased, regardless of increasing water intakes. Therefore, despite the presence of an interaction, it appeared that the effect on excreta moisture of dietary sodium and phosphorus at concentrations found in proprietary rations was linear. This additive response was similar to that found for dietary potassium and sodium (Hijikuro, 1976; Kando and Ross, 1962 b).

5.4.3. Effect of sodium source Experiment 5 demonstrated that the responses in excreta moisture and water intake of the hens were not ($p>0.05$) altered by the anion (chloride or bicarbonate) associated with the dietary sodium. This agrees with data of Damron *et al*, (1984), Damron, (1982) and Vogt, (1971) with broiler chickens and Davison and Wideman (1992) in layers, all reporting increased daily water intake and moisture content of faeces with sodium intake regardless of anion, although Leeson and Summers (1992) have produced conflicting data with laying hens.

5.5. CONCLUSION

In conclusion, the effect of increasing the dietary sodium, potassium or phosphorus concentration of the diet of laying hens was, to produce for each a linear increase in the moisture content of excreta. The effect was greater for potassium and sodium than phosphorus. Variation in dietary calcium had no effect on excreta moisture. Sodium and phosphorus acted in an additive manner, and the response to dietary sodium was not altered by two different anions (chloride and bicarbonate).

CHAPTER 6.

THE EFFECT OF DIETARY CRUDE PROTEIN CONCENTRATION, LYSINE AND METHIONINE CONCENTRATION, AND AMINO ACID AVAILABILITY ON EXCRETA MOISTURE AND WATER INTAKES OF LAYING HENS

6.1. INTRODUCTION

Considerable data on the effects of dietary crude protein concentration on water balance of the fowl have been reported (Eley and Hoffman, 1949; Glista and Scott, 1949; Patrick and Ferrise, 1962; Ward *et al,* 1975; Marks and Pesti, 1984; Cooke and Raine, 1986; Wheelhouse *et al,* 1985; Lopez, 1994). Conclusions on the exact effect are, however, equivocal, and may be incorrect, due to a failure of workers to distinguish between the effects of crude protein, and those of associated concentrations of major minerals (Mongin, 1989). Other ambiguities also exist, as workers have compared protein sources with differing levels of amino acid availability, and amino acid balance, as well as differing concentrations of non protein components which could effect water balance, (Pattison, 1989) and yet in each case explained their observations as an effect of increased crude protein concentration.

The effect of dietary crude protein concentration on water balance has been linked to increased nitrogen excretion, (Patrick and Ferrise, 1962; Lopez, 1994), although no direct evidence exists. Amino acids supplied in excess in the diet may not be stored for any length of time in the bird. Any increase in nitrogen intake increases both nitrogen retention and nitrogen excretion (Carr *et al,* 1977; Fernandez - Figares *et al,* 1995) through an increased amino acid oxidation (e.g. Soliman and Harper, 1971). Uric acid is the principle means of nitrogen excretion, and the major nitrogenous component of avian urine (Sturkie, 1965; Shoemaker, 1972). Synthesis is controlled by hepatic xanthine oxidase, the activity of which is increased by raised dietary protein. Any abnormal increase in the concentration of uric acid in blood could result in precipitation in the kidneys, joints and pericardium. It is possible that increased water intake and excretion will occur, in order to solubilise and excrete an excess of uric acid with an excess of dietary crude protein.

When excesses of individual amino acids are supplied in the diet, and maximal rates of protein

synthesis are obtained, the remaining amino acids are catabolized. Amino acid oxidation for most amino acids is proportional to the level of the amino acid in the diet (Vernon - Young *et al,* 1990). Increased catabolism of amino acids will raise the concentration of uric acid for excretion and therefore may have similar effects to increased crude protein concentration on water balance.

Jordan (1990) has reported that reduced amino acid availability can cause sticky droppings. Amino acids are polar molecules and if poorly absorbed in the small intestine may influence water reabsorption in the hindgut directly, or alternatively could influence the production of osmotically active volatile fatty acids produced by the microbial flora. Variation in the availability of amino acids may also effect the concentrations of uric acid excreted.

There is a need for information that quantitatively describes the variation in excreta moisture to differing concentrations of crude protein, which is free of the ambiguities incurred in previous work, and also for information describing the effects of variation in the concentrations of individual amino acids at constant crude protein concentration and variation in the amino acid availabilities of crude protein on excreta moisture.

Three separate experiments were carried out in this project to quantitatively measure the response in water intake and excreta moisture of laying hens to dietary concentrations of crude protein, when all amino acids were balanced in proportion to requirement, and to differing lysine and methionine concentrations at constant crude protein concentration whilst maintaining constant dietary concentrations of major minerals. A second objective of these experiments was to examine crude protein concentration x lysine concentration and methionine x lysine concentration interactions. The objective of a fourth experiment was to examine if excreta moistures varied when a series of diets containing a single level of crude protein, of identical quality but with differing levels of amino acid availability were fed.

For these experiments dried egg protein was used to provide crude protein in excess of requirements. This had numerous advantages; an amino acid balance which differed only slightly from the ideal amino acid balance for the laying hen, amino acids which were readily available, and a low potassium concentration (Appendix 6).

6.2. MATERIALS AND METHODS

6.2.1. Experiment 6 The objective of this experiment was to quantitatively measure the effect of varying dietary concentrations of a crude protein with an ideal amino acid balance on the excreta moisture of laying hens.

Forty eight, 38-week-old ISA Brown laying hens were caged in individual wire floored cages (50 x 45 x 45 cm), arranged in four tiers, within an environmentally controlled room. Each cage had an individual feed hopper, water trough and excreta collection tray. The birds were maintained under a 14L : 10D lighting regime at 24 ±1 °C and 80 ±5 % relative humidity.

Practical laying hen diets (11.80 MJ/kg of ME) were used that were identical in nutrient composition, except in crude protein concentration (Table 6.1.). The diets were formulated to contain six concentrations of crude protein (140, 160, 180, 200, 220 and 250 g/kg). Crude protein in excess of that in the basal diet was supplied from dried whole egg (Framptons Ltd, Shepton Mallet, Somerset, UK) (Appendix 6) and replaced washed sand and soyabean oil in the diet. Use of dried whole egg enabled each of the series of diets to provide differing crude protein concentrations, with available amino acids balanced in proportion to requirements of the National Research Council (1984) for laying hens. Macro mineral concentrations were maintained constant by replacement of washed sand with inorganic mineral salts. Each bird had *ad libitum* access to one of the six diets and water throughout the 8 d feeding period.

Water intakes and feed intakes were measured, and all excreta were collected for each 24 h period of the final two days of the feeding period. Excreta were collected in open trays and excreta moisture levels determined by drying total excreta at 60 °C in a forced air oven. Data were then corrected for loss of moisture to the environment, using the correction factor obtained in

Table 6.1. Composition (g/kg diet) and analysis of the basal diet for experiment 6

Component	g/kg	
Wheat	175.1	
Barley	69.8	
Sunflower meal	325.0	
Fish meal	20.0	
Lysine hydrochloride	8.0	
Methionine	6.0	
Soya oil[1]	154.0	
Limestone	87.5	
Dicalcium phosphate	0.5	
Sodium chloride	0.4	
Sand[1]	141.2	
Vitamin mineral premix[2]	12.5	
Analysis (calculated)		(g/kg crude protein)
Lysine	11.5	79.1
Methionine	8.9	59.3
Methionine + cysteine	10.2	68.7
Tryptophan	1.8	11.3
Threonine	4.8	82.6
Glycine + serine	11.7	80.3
Leucine	8.8	80.2
Isoleucine	6.0	41.3
Valine	7.3	48.7
Histidene	3.2	21.4
Argenine	10.5	71.4
Phenylalanine + tyrosine	9.5	64.6
Calcium	40.0	
Phosphorus	3.2	
Sodium	1.4	
Potassium	4.7	
Chloride	0.8	
Magnesium	2.0	
Crude protein	146.4	
ME (MJ of ME/kg)	11.8	

1. Washed sand and soya oil replaced for dried egg protein in the experimental diets at levels of 28.7, 71.0, 120.0 156.0 and 230.0g/kg
2. Comprised ash (890 g/kg), calcium (250 g/kg), methionine (80 g/kg), sodium (88.0 g/kg), copper (cupric sulphate 400 mg/kg), vitamin A (480000 i.u./kg), vitamin E alpha tocopherol acetate (480 i.u./kg), vitamin D3 (240000 i.u./kg)

Table 6.2. Composition (g/kg diet) and analysis of basal diets for experiment 7

	Diet			
	(160 g/kg crude protein)		(220 g/kg crude protein)	
Component				
Wheat	200.0		200.0	
Barley	100.0		100.0	
Meat and bone meal	30.0		30.0	
Lysine hydrochloride	5.0		5.0	
Methionine	1.0		1.0	
Tryptophan	0.5		0.5	
Soyabean oil	92.0		37.5	
Casein	20.5		20.5	
Limestone	87.5		87.5	
Dicalcium phosphate	10.0		10.0	
Sand[1]	192.0		134.0	
Dried whole egg	245.0		357.5	
Vitamin mineral premix[2]	12.5		12.5	
Analysis (calculated)	g/kg diet	g/kg crude protein	g/kg diet	g/kg crude protein
Lysine	16.0	95.0	20.0	95.0
Methionine	7.1	42.6	9.0	41.0
Methionine + cysteine	13.4	79.8	16.3	74.1
Tryptophan	2.8	16.9	3.7	16.7
Threonine	8.2	49.2	11.0	49.8
Glycine + serine	19.4	115.0	25.2	114.0
Leucine	15.9	94.8	21.0	95.4
Isoleucine	12.3	73.2	16.8	75.9
Valine	12.4	73.4	16.4	74.2
Histidene	3.9	23.4	5.1	22.8
Argenine	12.1	72.1	16.2	73.3
Phenylalanine + tyrosine	16.4	97.5	21.5	97.2
Calcium	44.3		44.3	
Phosphorus	4.0		4.0	
Sodium	1.5		1.5	
Potassium	1.5		1.6	
Chloride	0.7		0.7	
Magnesium	1.2		0.8	
Crude protein	160.0		220.0	
ME (MJ of ME/kg)	12.1		12.1	

1. Washed builders sand used as a filler in the diets of differing crude protein concentration
2. Comprised ash (890 g/kg), calcium (250 g/kg), methionine (80 g/kg), sodium (88 g/kg), copper (cupric sulphate 400 mg/kg) vitamin A (480000 i.u./kg), vitamin E alpha tocopherol acetate (480 i.u./kg), vitamin D3 (240000 i.u./kg)

experiment 1. Water and feed intakes were determined by weighing the appropriate trough at the beggining and the end of the collection period. Water intakes were adjusted for loss of water vapour from the drinkers. Only on egg laying days were data used in calculation of treatment means, to reduce variation as a result of a response in water balance to egg formation (Wood - Gush and Horne, 1970).

The experiment was designed as a randomised block analysis of variance, with cage tier level as a blocking factor. Treatment sums of squares were partitioned into a set of orthogonal linear and quadratic polynomial regression components using the GENSTAT statistical package (Lawes Agricultural Trust, 1984). Multiple regression analyses with water intake and feed intake as independent variables, and excreta moisture as a dependant variable were also carried out.

6.2.2. Experiment 7 The first objective of this experiment was to confirm the changes in excreta moisture and water intakes when two different concentrations of crude protein, with amino acids balanced in proportion to requirement, were present in laying hen rations. The second objective was to quantitatively measure the change in excreta moisture and water intake when six concentrations of lysine in excess of requirement were fed to laying hens at each dietary crude protein concentration, and the third was to examine whether there were any crude protein concentration x lysine concentration interactions.

The cage dimensions, and the environmental conditions for this experiment were the same as used in experiment 6. Twelve semi purified diets (Table 6.2.) that met all nutrient requirements of the laying hen, (12.10 MJ of ME/kg), were fed to ninety six, 38-week-old ISA Brown laying hens. The feeds provided two concentrations (160 or 220 g/kg) of crude protein as dried egg protein (with exception of 42% of the basal diet) and one of six concentrations of lysine (95, 125, 150, 175, 200 or 250 g/kg of crude protein) as L- Lysine HCL at each concentration of crude protein (Table 6.3.)

Table 6.3. Composition of experimental diets for experiment 7

		Lysine (g/kg crude protein)					
Crude protein	Composition (g/kg)	95	125	150	175	200	250
160 g/kg	Lysine HCl	5.0	11.9	18.0	23.6	29.4	41.2
	Soya oil[1]	92.0	97.0	100.0	104.0	107.5	113.0
	Dried egg protein[2]	245.0	231.0	220.0	208.0	196.0	172.0
	Sand	192.0	194.1	196.0	198.4	201.1	207.8
220 g/kg	Lysine HCl	5.0	15.5	23.2	30.8	38.7	54.0
	Soya oil[1]	37.5	44.0	48.0	52.5	55.0	65.0
	Dried egg protein[2]	357.5	334.5	320.0	304.0	289.5	258.0
	Sand	134.0	140.0	142.8	146.6	150.8	157.0

1. Soya oil added to maintain ME of diets with reduced dried egg protein concentration
2. Dried egg protein removed with increasing lysine concentration to maintain constant crude protein concentration

Dried egg protein and L- lysine HCL were added, in replacement for washed sand and soya oil. Basal 160 and 220 g/kg crude protein diets provided differing overall amino acid concentrations that were balanced in proportion to requirements of the National Research Council, (1984). Dietary crude protein concentrations were maintained constant at each concentration of lysine supplementation by removal of an appropriate portion of dried egg protein, and addition of soya oil, to maintain metabolisable energy concentrations. Macro mineral concentrations were maintained constant by replacement of washed sand with inorganic mineral salts. Each bird had *ad libitum* access to one of the six diets, and water throughout the 8 d feeding period.

The experiment was designed as a randomised block analysis of variance, with cage tier level as a blocking factor. Lysine concentration and lysine concentration x protein concentration interaction sums of squares were partitioned into a set of orthogonal linear and quadratic polynomial regression components, using the GENSTAT statistical package (Lawes Agricultural Trust, 1984). Multiple regression analyses, with water intake and feed intake as independent variables, and excreta moisture as a dependant variable, were also performed.

6.2.3. Experiment 8 The first objective of this experiment was to quantitatively investigate if dietary methionine concentration in excess of requirement had the same effect as lysine excess on excreta moisture and water intake, at constant dietary crude protein concentration, in laying hen rations. A second objective was to examine for any methionine concentration x lysine concentration interactions. A randomised block analysis of variance was used, arranged in a 4 x 4 factorial.

The cage dimensions and the environmental conditions were the same as used in experiment 6 and 7. Sixteen diets (Table 6.4.) that met all nutrient requirements of the laying hen (12.00 MJ/kg of ME and 171 g/kg of crude protein) were fed to sixty four, 42-week-old ISA Brown laying hens.

Table 6.4. Composition (g/kg diet) and analysis of the basal diet for experiment 8

Component	g/kg diet	
Wheat	175.1	
Barley	69.8	
Sunflower meal	300.0	
Fish meal	31.0	
Lysine Hydrochloride	7.0	
Methionine	5.0	
Tryptophan	0.5	
Soya oil[1]	142.0	
Limestone	87.5	
Dicalcium Phosphate	0.5	
Sodium chloride	0.4	
Dried egg protein[1]	55.0	
Sand[2]	113.7	
Vitamin mineral premix[3]	12.5	
Analysis (calculated)		(g/kg crude protein)
Lysine	13.0	76.1
Methionine	8.9	51.9
Methionine + cysteine	10.9	63.4
Tryptophan	2.4	14.0
Threonine	14.7	85.7
Glycine + serine	13.9	80.9
Leucine	10.9	63.8
Isoleucine	8.2	47.8
Valine	9.3	54.1
Histidene	3.7	21.4
Argenine	12.3	71.7
Phenylalanine + tyrosine	11.6	67.9
Calcium	40.1	
Phosphorus	4.1	
Sodium	1.4	
Potassium	4.5	
Chloride	0.8	
Magnesium	2.00	
Crude protein	171.3	
ME (MJ of ME/kg)	12.0	

1. Dried egg protein removed in formation of experimental rations with increasing lysine/ methionine to maintain crude protein concentration
2. Washed sand replaced for methionine and lysine in formulation of experimental rations as described in text
3. Comprised ash (890 g/kg), calcium (250 g/kg), methionine (80 g/kg), sodium (88 g/kg), copper (cupric sulphate 400 mg/kg), vitamin A (480000 i.u./kg), vitamin E alpha tocopherol acetate (480 i.u./kg), vitamin D3 (240000 i.u./kg)

The basal diet provided each amino acid balanced in proportion to National Research Council (1984) requirements for laying hens. Addition of one of four concentrations of lysine (76.0, 87.5, 100.0 and 130.0 g/kg of crude protein) as L-lysine HCL and one of four concentrations of methionine (52.0, 75.0, 87.5 and 100.0 g/kg of crude protein) as DL-methionine, both in replacement for washed sand, and an appropriate amount of dried egg to maintain constant dietary crude protein concentrations, produced the sixteen diets. Macro mineral concentrations were maintained constant by replacement of washed sand as for experiment 6.

The experiment was designed as a randomised block analysis of variance, with cage tier level as a blocking factor. Lysine, methionine and lysine concentration x methionine concentration interaction sums of squares were partitioned into a set of orthogonal linear and quadratic polynomial regression components, using the GENSTAT statistical package (Lawes Agricultural trust, 1984). Multiple regression analyses, with water intake and feed intake as independent variables, and excreta moisture as a dependent variable were also carried out.

6.2.4. Experiment 9 The first objective of this experiment was to quantitatively measure the change in excreta moisture and water intake of laying hens (42 weeks old) when fed dietary crude protein of consistent amino acid composition, but with differing availability. A second objective was to examine for changes in weight and moisture content of caecal and colonic digesta, and, or, changes in ceacal and colonic tissue wet weight with different levels of dietary amino acid availability.

The cage dimensions and environmental conditions were the same as used in experiments 6, 7 and 8. Six diets were compared (Table 6.5.). All diets contained 169.5 g of crude protein/kg of which 140 g/kg comprised de-hulled soya bean meal (485 g crude protein/kg DM) in differing combinations (0:100, 20:80, 40:60, 60:40, 80:20 and 100:0) of a severely heat treated (see 6.2.5),

Table 6.5. Composition (g/kg diet) and analysis of basal diet for experiment 9.

Component	g/kg	
Wheat	300.0	
Barley	284.6	
De-huled soyabean meal[1]	225.0	
Soya oil	75.0	
Limestone	87.5	
Dicalcium Phosphate	15.0	
Sodium chloride	0.4	
Vitamin mineral premix[2]	12.5	
Analysis (calculated)		(g/kg crude protein)
Lysine	8.8	52.0
Methionine	3.5	20.7
Methionine + cysteine	5.8	34.2
Tryptophan	2.1	12.3
Threonine	6.2	88.1
Glycine + serine	15.3	89.8
Leucine	12.9	78.0
Isoleucine	7.7	45.4
Valine	8.5	50.1
Histidene	4.0	23.7
Argenine	11.0	65.2
Phenylalanine + tyrosine	18.9	82.5
Calcium	42.8	
Phosphorus	4.5	
Sodium	1.4	
Potassium	7.4	
Chloride	0.8	
Magnesium	1.3	
Crude protein	169.5	
ME (MJ of ME/kg)	11.9	

1. Comprised either a severely heat treated or mildly heat treated form or a blend of the two soya bean meals as described in text
2. Comprised ash (890 g/kg), calcium (250 g/kg), methionine (80 g/kg), sodium (88 g/kg), copper (cupric sulphate 400 mg/kg), vitamin A (480000 i.u./kg), vitamin E alpha tocopherol acetate (480 i.u./kg), vitamin D3 (240000 i.u./kg)

and mildly heat treated form (see 6.2.5), producing six diets of decreasing amino acid availability (Table 6.6.). Diet formulations were adjusted for differences in dry matter contents of each soyabean meal (880 and 950 g DM/kg non heat treated and heat treated de-hulled soyabean meals respectively) using washed sand.

Measurements taken were the same as in experiments 6, 7 and 8, except after eight days excreta were also scored according to their consistency. The scoring system placed excreta into one of six divisions from pasty (1) to particulate (6). Secondly eight birds (four birds fed 100% heat treated de-hulled soyabean meal and four fed 100% non heat treated de-hulled soyabean meal) were weighed and slaughtered using a 0.5 ml intravenous dose of sodium pentobarbitone to avoid disturbance in digesta distribution through contraction of colonic and caecal musculature. Caeca and colon were removed separately below the ileo-colonic caecal junction, and the small intestine between Merckel's diverticulum and the ileo-colonic-caecal junction, and were weighed. Digesta were removed by manual compression, weighed, and dried at 60 °C to determine moisture content. Caecal and colonic tissue were washed and weighed. All data were adjusted for variation in body weight.

6.2.5. Preparation and assay of heat treated soyabean meal Heat treatment to reduce amino acid availability of the de-hulled soya bean meal followed the procedure of Hayes *et al*, (1983) which involved autoclaving at 121 °C and 115 psi for 15 h. Ten adult ISA Brown cockerels were used to measure the availability of amino acids in ground samples, (50 g), of both heat treated and non heat treated de-hulled soya bean meal, in a true available amino acid (TAAA) assay, according to a modified procedure for true metabolisable energy described by Mc Nab and Blair (1988) (Appendix 11). Two similar birds were given 50 g of sucrose; these served as controls, and their excreta were used to determine endogenous amino acid and nitrogen loss. For amino acid

Table 6.6. Amino acid composition and availability of de-hulled soyabean meal before and after heat treatment

	Non heat treated soya bean meal[1]			Heat treated soya bean meal[2]					
	Total amino acid concentration[3] (g/kg diet)	g/kg crude protein	% availability[4]	Total amino acid concentration[3] (g/kg)	g/kg crude protein	% availability[4]	% decrease	SEM	Significance[5]
Amino acid									
Aspartic acid	57.20	27.74	90.91±2.05	55.50	26.94	60.22±5.46	33.80	4.130	***
Threonine	21.40	10.38	89.56±2.55	21.20	10.28	60.85±5.61	32.10	4.360	***
Serine	27.70	13.43	91.66±1.99	28.00	13.58	67.28±4.47	26.60	3.580	***
Glutamic acid	96.40	46.75	92.55±2.12	95.10	46.12	66.50±5.27	28.15	4.010	***
Proline	28.80	13.97	92.18±2.41	28.50	13.82	63.55±3.98	31.06	3.350	***
Alanine	22.10	10.72	87.33±2.98	22.50	10.91	59.47±7.43	31.90	5.660	***
Cystine	6.60	3.20	89.27±4.08	5.10	2.47	57.25±10.20	35.87	7.780	***
Valine	21.40	10.38	88.16±2.97	21.10	10.23	59.71±4.70	32.28	3.940	***
Methionine	5.50	2.67	91.92±3.59	5.20	2.52	66.53±4.00	27.63	3.800	***
Isoleucine	21.50	10.42	90.25±2.53	20.70	10.04	63.77±4.74	29.35	3.800	***
Leucine	38.10	18.47	91.35±2.19	37.50	18.19	68.21±4.83	25.34	3.160	***
Tyrosine	19.80	9.60	95.11±2.75	19.10	9.26	71.62±6.37	24.70	4.910	***
Phenylalanine	17.20	8.34	89.98±3.04	26.60	12.90	61.89±4.06	28.09	3.590	***
Histidine	13.50	6.55	91.41±1.12	12.70	6.15	64.67±4.39	29.25	3.200	***
Lysine	29.30	14.21	91.20±1.86	25.80	12.51	67.16±1.76	26.36	1.809	***
Arginine	40.50	19.64	96.04±0.90	28.10	13.63	65.69±4.06	31.19	2.943	***

1. 'Non heat treated' soyabean meal was heated during commercial preperation to remove the heat labile anti- trypsin factor
2. Heat treated soyabean meal was prepared by autoclaving for 15 h at 121 °C (Hayes *et al*, 1983)
3. Determined by ion exchange chromatography
4. Mean ± standard deviation amino acid availability determined by total available amino acid (TAAA) assay with 5 cockerels per determination
5. Significance of decrease in amino acid availability *** ($p<0.001$)

analysis samples of each of the de-hulled soya bean meals, and excreta were hydrolysed with 6N HCl at 110 °C for 24 h. Amino acid analysis of the hydrolysate was carried out by ion exchange chromatography (A.O.A.C, 1990). True amino acid availability was calculated for each amino acid using the following equation:-

$$\% \text{ TAAA} = \frac{\text{AA intake} - (\text{AA excretion} - \text{AA}_{\text{Mf}+\text{Eu}}) \times 100}{\text{AA intake}}$$

Where AA_{MF+EU} = Amino acid excretion of unfed birds

Nitrogen and uric acid concentration were determined according to the methods described below.

*6.2.6. **Feed and excreta analyses*** Samples of feeds for each experiment were ground to pass a 0.5 mm mesh screen, and were subsequently analysed by standard proximate analysis (A.O.A.C., 1990). Sodium, calcium, phosphorus and potassium concentrations were determined with an atomic absorption spectrophotometer (Smith-Hieftje 1000, Thermo Jarrell Ash Corporation) following wet digestion (A.O.A.C., 1990). Inorganic phosphorus was determined, using a colorimetric technique (MAFF, 1986) in which concentration of phosphorus in a trichloroacetic acid extract is determined spectrophotometrically as the yellow phospho - vanado - molybdate complex at 400 nM. Diets which failed to conform to the calculated analysis +/- 10% (Tables 6.1, 6.2 and 6.4) were re-mixed and re-analysed.

Total excreta following drying were ground to pass a 0.5 mm mesh screen. Samples were subsequently analysed for Nitrogen by the Kjeldahl method (A.O.A.C. 1990) using a Kjeltec autoanalyser and, uric acid concentration by way of uricase and differential spectrophotometry as

described by Pudelkiewicz *et al*, (1969) (Appendix 4).

6.2.7. Blood serum analysis Blood samples were removed by puncture of the wing vein of each bird approximately six hours after the start of the light period, on the final day of each experiment. Samples were allowed to clot, centrifuged at 1500 g for 15 minutes, and serum removed. Samples were frozen at -20 ±1 °C until assayed. Serum was analysed for uric acid concentration, using a Technicon RA-1000 blood analyser according to the method of Miles inc (Appendix 3). Only data from birds which had laid an egg that day were used in calculation of treatment means, to reduce variation due to differences in blood volume with variation in water balance with egg formation (Howard, 1975).

6.3. RESULTS

6.3.1. Experiment 6 (see Tables 6.7 and 6.8.) There was no effect of dietary crude protein concentration on feed intake ($p>0.05$), so increased dietary crude protein concentration produced a linear increase ($p<0.001$) in crude protein intake. Increased dietary crude protein concentration increased water intakes ($p<0.001$) (Figure 6.1.) and water to feed intake ratios ($p<0.001$). There were also linear increases ($p<0.001$) in the weights of water excreted, and the moisture contents of the excreta (g/kg) (Figure 6.1.), but no effects on total outputs (fresh weight) of excreta ($p>0.05$). Increased dietary crude protein concentration gave linear increases ($p<0.001$) in nitrogen intake, nitrogen absorbed, nitrogen retained, serum uric acid concentration, total nitrogen, uric acid nitrogen excretion and the ratio of uric acid nitrogen to nitrogen in excreta. There were no effects on faecal nitrogen excretion ($p>0.05$). There were no effects ($p>0.05$) of crude protein concentration on the sodium (1.480 ±0.127g/kg), potassium (4.92 ±0.174 g/kg) or phosphorus (3.54 ±0.236 g/kg) concentration of the diets

There were positive linear relationships ($p<0.001$) between excreta moisture of the hens and the concentrations of uric acid nitrogen ($r = 0.82$), the ratio of uric acid nitrogen to total nitrogen ($r = 0.74$) in the excreta, concentrations of uric acid in blood serum ($r = 0.69$), the amount of nitrogen absorbed ($r = 0.67$) and total nitrogen in excreta ($r = 0.40$) ($p<0.01$). There were however no correlations ($p>0.05$) with faecal nitrogen.

6.3.2. Experiment 7 (see Table 6.9 and 6.10.). Increased crude protein concentration raised ($p<0.001$) excreta moisture and water intakes, nitrogen intake, nitrogen absorbed, total nitrogen, uric acid nitrogen and the ratio of uric acid nitrogen to total nitrogen in excreta consistent with the previous experiment.

Table 6.7. The effect of dietary crude protein concentration on the excreta moisture, water intake and other parameters of laying hens

	Dietary crude protein (g/kg)							
	139.90	160.60	180.20	201.30	220.10	250.10	SEM[1]	Significance[2]
Excreta moisture (g/kg)	590.20	625.80	659.40	670.80	699.30	742.10	35.400	***
Water intake (g/b/day)	308.10	287.50	324.10	336.70	364.20	372.50	50.130	***
Food intake (g/b/day)	207.50	236.50	225.40	217.80	208.20	196.40	31.010	NS
Water: feed intake (g/g)	1.49	1.23	1.52	1.56	1.75	1.91	0.303	***
Total excreta (g/b/day)	185.20	198.00	211.60	223.80	202.40	215.00	27.450	NS
Water excreted (g/b/day)	109..40	124.40	139.90	151.00	141.60	159.50	22.100	***
Dry matter excreted (g/b/day)	75.85	73.68	71.70	72.80	60.70	55.50	9.720	***

1. Error df = 39 (all parameters)
2. Significance level of the slope (b) where y=a+bx *** ($p<0.001$), ** ($p<0.01$), * ($p<0.05$), NS ($p>0.05$)

Table 6.8. The effect of dietary crude protein concentration on nitrogen metabolism of laying hens

	Dietary crude protein (g/kg)							
	139.90	160.60	180.20	201.30	220.10	250.10	SEM[1]	Significance[2]
Nitrogen intake (g/b/day)	4.62	6.08	6.49	7.01	7.33	7.85	0.897	***
Nitrogen absorbed[3] (g/b/day)	3.48	4.91	5.09	5.36	6.17	6.21	0.948	***
Nitrogen excreted[3] (g/b/day)	1.79	2.01	2.63	3.31	2.93	3.50	0.513	***
Nitrogen retention[3] (g/b/day)	2.83	4.07	3.86	3.70	4.39	4.35	0.847	***
Efficiency of N utilisation (%)	81.20	82.60	74.30	69.70	71.30	69.50	6.950	**
Uric acid excreted (g/b/day)	1.97	2.55	3.71	5.04	5.39	5.63	1.233	***
Uric acid nitrogen (g/b/day)	0.649	0.842	1.22	1.66	1.78	1.86	0.402	***
Faecal nitrogen[3] (g/b/day)	1.14	1.17	1.41	1.65	1.16	1.64	0.683	NS
Uric N /total N (%)	36.9	41.90	48.70	50.70	63.30	55.80	15.610	**
Serum uric acid (umol/l)	323.00	392.00	408.10	567.60	693.10	701.30	22.100	***

1. Error df = 39 (all parameters)
2. Significance level of the slope (b) where y=a+bx *** ($p<0.001$), ** ($p<0.01$), * ($p<0.05$), NS ($p>0.05$)
3. Nitrogen absorption, retention, faecal nitrogen and nitrogen excretion are apparent values as no determination of endogenous losses were made

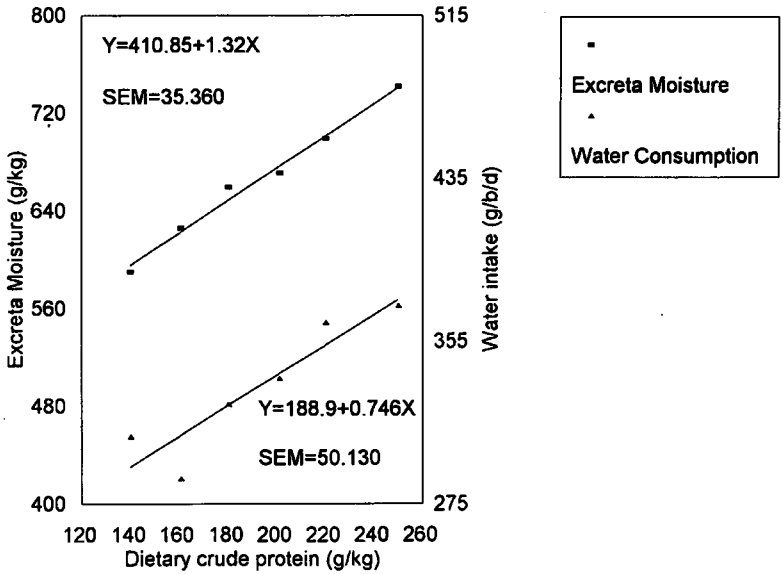


Figure 6.1. The effect of dietary crude protein concentration on the excreta moisture and water intake of laying hens

There was a linear decrease ($p<0.01$) in feed intake with increased dietary lysine concentrations. However increased dietary lysine concentrations still gave linear increases in daily lysine intake ($p<0.001$), water intakes ($p<0.05$) (Figure 6.3.) and water to feed intake ratios ($p<0.01$). There were therefore linear increases ($p<0.01$) in the moisture contents of the excreta (g/kg) (Figure 6.2.), but there were no effects on total output of excreta ($p>0.05$). Increased dietary lysine concentration gave linear decreases in ($p<0.01$) nitrogen intake, nitrogen absorbed and nitrogen retention. However, increased dietary lysine concentration also gave linear increases in serum uric acid concentration ($p<0.001$), total nitrogen ($p<0.05$), uric acid nitrogen ($p<0.01$) and the ratio of uric acid nitrogen to total nitrogen in excreta ($p<0.05$), but had no effect on faecal nitrogen ($p>0.05$).

There were protein concentration x lysine concentration interactions for water intake ($p<0.05$) and the ratio of uric acid nitrogen to total nitrogen excreted ($p<0.01$), but there were no interactions ($p>0.05$) for any of the other parameters measured.

There were positive correlations ($p<0.001$) between the excreta moisture of the hens, and the concentration of uric acid nitrogen ($r= 0.50$), the ratio of uric acid nitrogen to total nitrogen in the excreta ($r = 0.53$), total nitrogen in excreta ($r = 0.31$) and in concentrations of uric acid in blood serum ($r= 0.64$), but there were no correlations ($p>0.05$) with faecal nitrogen.

There were no effects ($p>0.05$) of crude protein or lysine concentration on the sodium (1.65± 0.412 g/kg), potassium (1.87± 0.129 g/kg) or phosphorus concentration of the diets (3.82± 0.143 g/kg).

6.3.3. Experiment 8 (see Table 6.11 and 6.12 a and b.)There were linear decreases ($p<0.01$) in

intakes with increased dietary lysine concentrations, however increased dietary lysine concentrations still gave linear increases ($p<0.001$) in daily lysine intakes, water intakes, water to feed intake ratios, and moisture contents of excreta (g/kg) consistent with the previous experiment. The increased dietary lysine concentrations gave linear decreases ($p<0.01$) in nitrogen intake, absorption and retention and linear increases ($p<0.05$) in uric acid nitrogen excretion and serum uric acid concentration. In contrast to the previous experiment however there were no effects ($p>0.05$) on nitrogen excretion or the ratio of uric acid nitrogen to total nitrogen in excreta due to large treatment standard errors, and a smaller lysine range used. However the estimate of the regression coefficients for each parameter were not different ($p>0.05$) from the previous experiment.

There was a large linear decrease ($p<0.001$) in feed intake with increased dietary methionine concentrations. However increased dietary methionine concentrations still gave linear increases ($p<0.01$) in daily methionine intakes. Increased dietary methionine concentration also gave linear decreases ($p<0.001$) in water intakes, and therefore there were no effects ($p>0.05$) on the water to feed intake ratios. There were linear decreases ($p<0.001$) in the weights of water and dry matter excreted, and total outputs (fresh weight) of excreta, and therefore there were no effects of increased dietary methionine concentration on the moisture content (g/kg) of the excreta. Increased dietary methionine concentrations gave linear decreases in nitrogen excretion ($p<0.05$), serum uric acid concentration ($p<0.05$), uric acid nitrogen excretion ($p<0.001$) and the ratio of uric acid nitrogen to total nitrogen excretion ($p<0.001$).

There were no ($p>0.05$) methionine concentration x lysine concentration interactions for any of the parameters measured.

There were no effects ($p>0.05$) of variation in lysine or methionine concentration on the sodium

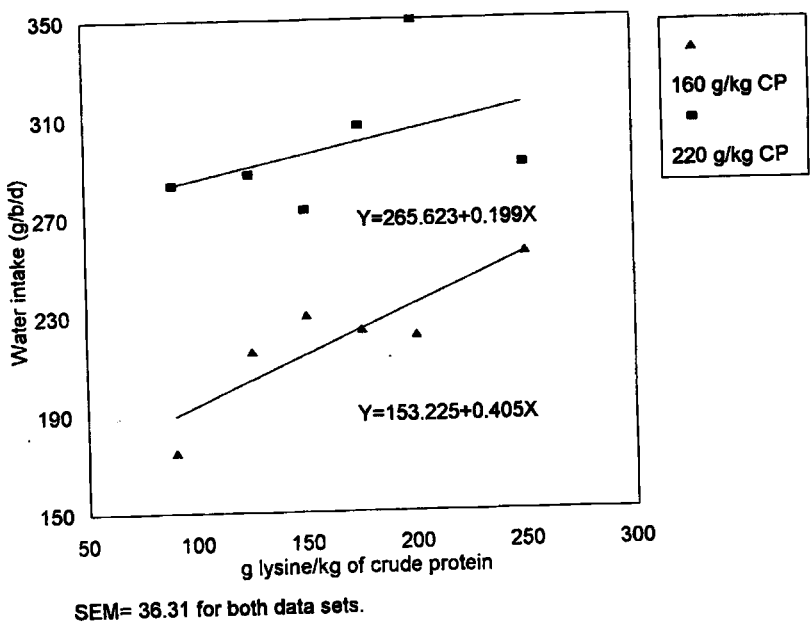


Figure 6.2. The effect of dietary lysine concentration at two different crude protein concentrations (160 and 220 g/kg) on water intake of laying hens

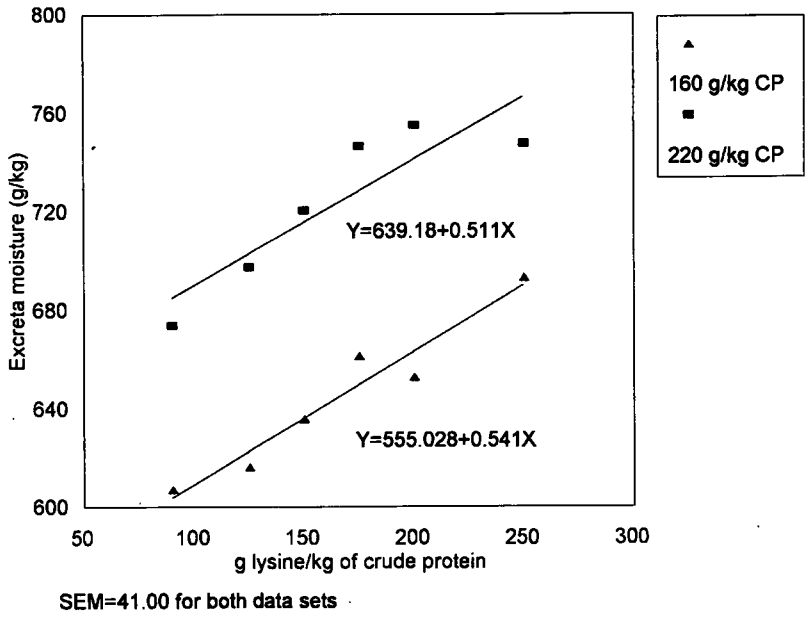


Figure 6.3. The effect of dietary lysine concentration at two different crude protein concentrations (160 and 220 g/kg) on excreta moisture of laying hens

Table 6.9. Effect of dietary lysine concentration at two differing crude protein concentrations on excreta moisture and water intakes of laying hens

	Dietary crude protein (g/kg)															
	160						220							Significance[2]		
g lysine/kg of CP	90	125	150	175	200	250	90	125	150	175	200	250	SEM[1]	Protein	Lysine	Protein x Lysine
Excreta moisture (g/kg)	607.20	616.10	635.60	661.30	652.70	693.20	673.80	697.50	720.60	746.50	754.90	747.40	41.000	***	***	NS
Water intake (g/b/day)	174.80	215.40	230.00	223.70	221.50	254.90	283.20	287.50	273.00	306.90	349.60	290.80	36.610	***	***	*
Food intake (g/b/day)	110.10	123.00	111.10	97.20	86.90	68.70	122.40	121.70	109.70	123.40	106.20	85.7	22.960	**	***	NS
Water: feed ratio (g/g)	1.76	1.76	2.11	2.39	2.67	4.22	2.36	2.45	2.60	2.59	3.60	3.50	0.838	***	***	NS
Total excreta (g/b/day)	72.30	67.60	78.20	78.30	72.60	83.70	87.70	88.50	87.10	100.30	92.80	102.40	14.990	***	**	NS
Water excreted (g/b/day)	43.90	41.80	49.60	51.80	47.50	58.00	59.20	61.70	62.60	74.71	70.10	76.70	11.090	***	***	NS
Dry matter excreted (g/b/day)	28.47	25.82	28.57	26.50	25.04	25.70	28.50	26.50	24.56	25.62	22.66	25.95	5.780	NS	NS	NS

1. Error df = 81 (all parameters)
2. Significance ***($p<0.001$), ** ($p<0.01$), * ($p<0.05$), NS ($p>0.05$)

Table 6.10. Effect of dietary lysine concentration at two differing crude protein concentrations on nitrogen metabolism of laying hens

	Dietary crude protein (g/kg)															
	160						220							Significance[2]		
g lysine/kg of CP	90	125	150	175	200	250	90	125	150	175	200	250	SEM[1]	Protein	Lysine	Protein x Lysine
Nitrogen intake (g/b/day)	2.85	3.15	2.84	2.49	2.23	1.76	4.31	4.30	3.86	4.34	3.74	3.02	0.725	***	***	NS
Nitrogen absorbed (g/b/day)[3]	1.98	2.41	2.05	1.81	1.44	1.05	3.49	3.46	3.09	3.53	2.90	2.07	0.755	***	***	NS
Nitrogen excreted (g/b/day)[3]	1.17	1.1	1.37	1.42	1.45	1.46	1.58	1.60	1.54	1.64	1.64	1.87	0.369	***	**	NS
Nitrogen retention (g/b/day)[3]	1.67	2.05	1.47	1.06	0.77	0.29	2.73	2.68	2.33	2.69	2.09	1.14	0.789	***	***	NS
Efficiency of N utilisation (%)[4]	69.70	76.40	65.90	61.70	50.80	54.60	76.30	76.50	74.70	76.70	72.60	57.90	46.940	***	**	*
Uric acid excreted (g/b/day)	0.97	1.10	1.73	2.26	2.02	2.30	2.29	2.37	2.36	2.53	2.46	2.81	0.568	***	***	NS
Uric acid nitrogen (g/b/day)	0.32	0.36	0.57	0.75	0.67	0.76	0.76	0.78	0.78	0.83	0.81	0.93	0.187	***	***	NS
Faecal nitrogen (g/b/day)[3]	0.85	0.74	0.79	0.68	0.79	0.71	0.82	0.83	0.77	0.81	0.83	0.95	0.237	NS	NS	NS
Uric N/total N (%)	47.50	49.40	49.70	49.60	48.30	47.30	47.50	49.50	49.70	49.60	48.30	47.30	6.740	***	***	**
Serum uric acid (µmol/l)	393.00	492.00	548.00	624.00	591.00	648.00	696.00	723.00	736.00	854.00	879.00	852.00	19.000	***	***	NS

1. Error df=81 (all parameters)
2. Significance ***(p<0.001), ** (p<0.01), * (p<0.05), NS (p>0.05)
3. Apparent values
4. Calculated as Nitrogen retained/ Nitrogen absorbed x 100

Table 6.11. The effect of dietary concentrations of methionine and lysine and their interactions on excreta moisture, water intake and some other parameters of laying hens

Diet		Parameter						
Lys (g/kg cp)	Met (g/kg cp)	Excreta Moisture (g/kg)	Water intake (g/b/day)	Food intake (g/b/day)	Water: feed ratio (g/g)	Total excreta (g/b/day)	Water excreted (g/b/day)	Dry matter excreta (g/b/day)
75.30	58.37	610.00	209.40	118.80	1.53	119.80	73.20	46.01
75.30	72.97	648.90	208.70	104.90	1.35	111.90	72.40	39.47
75.30	87.56	589.80	193.10	84.70	1.09	91.40	54.20	37.21
75.30	102.15	599.80	151.90	77.30	1.00	65.20	39.30	25.87
87.56	58.37	642.80	228.60	106.70	1.60	117.00	75.40	41.58
87.56	72.97	645.30	211.90	101.70	1.53	120.00	77.10	42.92
87.56	87.56	655.00	202.00	87.20	1.31	108.50	71.50	36.96
87.56	102.15	628.30	167.60	74.80	1.12	64.90	40.90	24.06
102.15	58.37	659.10	242.00	106.90	1.87	127.90	84.30	43.60
102.15	72.97	638.90	222.00	100.40	1.76	126.20	80.70	45.49
102.15	87.56	657.60	252.30	104.60	1.83	116.40	76.60	39.85
102.15	102.15	641.40	164.10	70.50	1.23	72.50	46.90	25.59
132.00	58.37	669.20	246.60	98.00	2.21	132.90	90.40	42.51
132.00	72.97	661.00	220.30	78.70	1.77	127.30	84.20	43.17
132.00	87.56	658.70	255.70	98.40	2.21	111.90	73.70	38.21
132.00	102.15	668.00	186.70	64.90	1.46	76.50	51.10	25.39
SEM[1]		37.590	31.160	10.300	0.366	23.310	16.920	7.710
Significance[2]	Met	NS	***	***	NS	***	***	***
	Lys	***	**	**	***	NS	**	NS
	Met x Lys	NS	NS	NS	NS	NS	NS	NS

1. Error df = 45 (all parameters)
2. Significance *** (p<0.001), ** (p<0.01), * (p<0.05), NS (P>0.05)

Table 6.12 a. The effect of dietary concentrations of methionine and lysine and their interactions on nitrogen metabolism in laying hens

Diet		Parameter				
Lys (g/kg cp)	Met (g/kg cp)	Nitrogen intake (g/b/day)	Nitrogen absorbed (g/b/day)[3]	Nitrogen excreted (g/b/day)[3]	Nitrogen retention (g/b/day)[3]	Efficiency of N utilisation (%)[4]
75.30	58.37	3.25	2.51	1.05	2.20	87.55
75.30	72.97	2.88	2.28	0.89	1.98	86.22
75.30	87.56	2.32	1.69	0.93	1.39	81.75
75.30	102.15	2.12	1.53	0.82	1.30	84.51
87.56	58.37	2.92	2.11	1.18	1.74	82.47
87.56	72.97	2.79	2.06	1.05	1.73	83.40
87.56	87.56	2.39	1.65	1.04	1.35	80.18
87.56	102.15	2.05	1.39	0.89	1.16	81.29
102.15	58.37	2.93	2.06	1.23	1.70	81.77
102.15	72.97	2.87	2.16	1.07	1.68	83.33
102.15	87.56	2.75	2.04	1.04	1.82	83.82
102.15	102.15	1.93	1.21	0.96	0.97	76.90
132.00	58.37	2.69	1.87	1.23	1.45	77.05
132.00	72.97	2.70	1.89	1.18	1.52	80.42
132.00	87.56	2.16	1.35	1.16	1.00	74.00
132.00	102.15	1.78	1.03	1.01	0.77	73.14
SEM[1]		0.282	0.291	0.267	0.325	7.174
Significance[2]	Met	***	***	*	***	NS
	Lys	**	**	NS	***	**
	Met x Lys	*	NS	NS	*	NS

1. Error df = 45 (all parameters)
2. Significance *** (p<0.001), ** (p<0.01), * (p<0.05), NS (P>0.05)
3. Apparent values as no determinations of endogenous losses were made
4. Calculated as nitrogen retained/ nitrogen absorbed x 100

Table 6.12 b. The effect of dietary concentrations of methionine and lysine and their interactions on nitrogen metabolism in laying hens

Diet		Parameter				
Lys (g/kg cp)	Met (g/kg cp)	Uric acid excreted (g/b/day)	Uric acid nitrogen (g/b/day)	Faecal nitrogen (g/b/day)[3]	Uric N/total N (%)	Serum uric acid (umol/l)
75.30	58.37	0.94	0.31	0.74	29.94	447.90
75.30	72.97	0.91	0.29	0.60	33.16	414.00
75.30	87.56	0.92	0.30	0.63	33.42	359.80
75.30	102.15	0.70	0.23	0.59	28.18	331.40
87.56	58.37	1.12	0.37	0.82	32.61	463.00
87.56	72.97	0.99	0.33	0.73	31.23	391.40
87.56	87.56	0.92	0.30	0.74	28.89	368.30
87.56	102.15	0.71	0.23	0.66	26.96	339.40
102.15	58.37	1.12	0.37	0.86	31.11	468.80
102.15	72.97	1.08	0.36	0.71	33.45	458.30
102.15	87.56	1.03	0.34	0.74	32.51	415.80
102.15	102.15	0.71	0.24	0.72	25.53	367.90
132.00	58.37	1.27	0.42	0.82	34.82	482.80
132.00	72.97	1.13	0.37	0.80	31.55	423.60
132.00	87.56	1.05	0.35	0.81	30.23	382.50
132.00	102.15	0.79	0.26	0.74	25.89	379.70
SEM[1]		0.219	0.072	0.223	5.647	36.140
Significance[2]	Met	***	***	NS	**	***
	Lys	**	**	NS	NS	*
	Met x Lys	NS	NS	NS	NS	NS

1. Error df = 45 (all parameters)
2. Significance *** (p<0.001), ** (p<0.01), * (p<0.05), NS (P>0.05)
3. Apparent values as no determinations of endogenous losses were made

(1.602± 0.209 g/kg), potassium (5.10± 0.143 g/kg) or phosphorus (3.89± 0.136 g/kg) concentrations of diets.

6.3.4. Experiment 9 True amino acid availability (TAAA) experiment The availability of all amino acids in the de-hulled soyabean meal measured, decreased ($p<0.001$) with heat treatment (Table 6.6.). There were therefore decreases ($p<0.001$) in nitrogen absorption, uric acid nitrogen excretion and the proportion of total nitrogen excreted as uric acid, and increases ($p<0.001$) in faecal nitrogen excretion (Table 6.13.)

Laying hen experiment (see Table 6.14. and 6.15.). There were no effects of replacing non heat treated soyabean meal with increasing dietary concentrations of the heat treated form on feed intakes ($p>0.05$), so increased concentrations of heat treated soyabean produced linear decreases ($p<0.001$) in predicted amino acid availability for each amino acid measured. Reduced amino acid availability had no effects ($p>0.05$) on nitrogen intakes, but reduced nitrogen absorption ($p<0.05$), and retention, serum uric acid concentrations ($p<0.05$), uric acid nitrogen excretion ($p<0.001$) and the ratio of uric acid nitrogen to total nitrogen excreted ($p<0.001$) and produced a linear increase ($p<0.05$) in faecal nitrogen excretion.

Reduced dietary amino acid availabilities reduced water intakes ($p<0.001$) (Figure 6.4.), and water to feed intake ratios ($p<0.05$). There were also linear decreases ($p<0.001$) in the weights of water excreted, the moisture contents of excreta (g/kg) (Figure 6.4.) and total outputs (fresh weight of excreta). Reduced amino acid availability produced a transient visually observable effect on the consistency of excreta (Figure 6.5.) for both cockerels, in the true amino acid availability assay, and in the laying hen trial. In the latter the effect was diminished by day four of the experiment. There were therefore no differences in excreta score ($p>0.05$) observable by day eight.

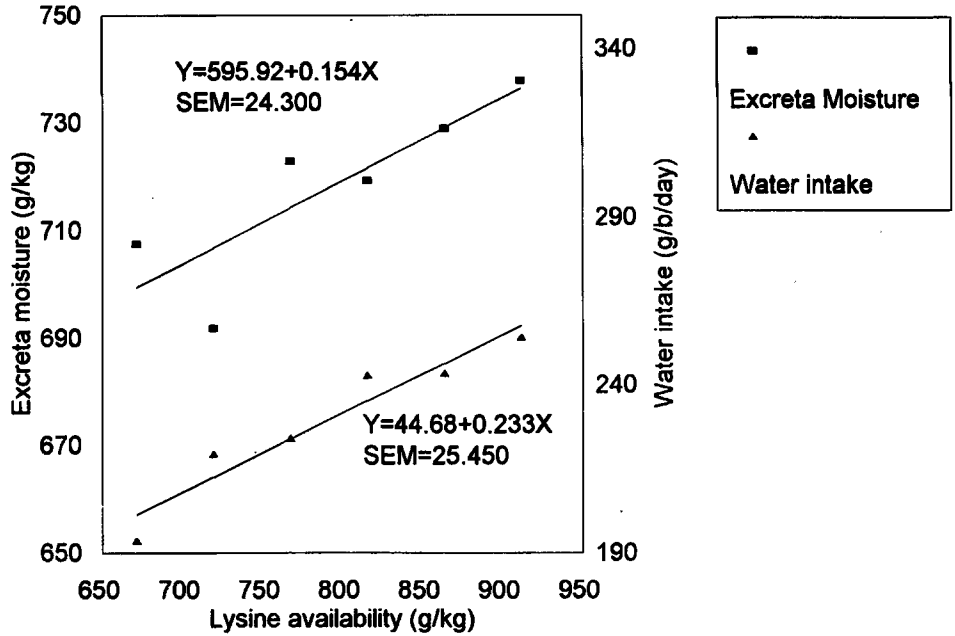


Figure 6.4[1].The effect of dietary lysine availability on excreta moisture and water intakes of laying hens

1. Linear estimates were derived using availability of dietary lysine whereas the true response observed was due to variation in availability of all amino acids in the diets. Lysine was used as it is easily analysed for, is an essential amino acid, particularly sensitive to heat treatment due to the ϵ-NH_2 group and is often first or second limiting in poultry diets and raw ingredients

Table 6.13. The nitrogen metabolism of cockerels fed heat treated or non heat treated soyabean meal

	Soyabean meal			
Parameter	Non heat treated	Heat treated	SEM[1]	Significance[2]
Nitrogen intake (g/b/48 h)	3.80	3.80	.....	NS
Nitrogen absorbed (g/b/48 h)	2.79	2.24	0.256	***
Nitrogen excreted (g/b/48 h)	2.78	2.93	0.388	NS
Nitrogen availability (g/b/48 h)	1.02	0.87	0.388	NS
Uric acid nitrogen (g/b/48 h)	1.77	1.37	0.192	***
Uric N/ total N (g/kg)	642.00	470.00	45.300	***
Faecal nitrogen (g/b/48 h)	1.01	1.56	0.256	***
Efficiency of N utilisation (%)[3]	35.60	38.10	11.24	NS

1. Error df = 8 (all parameters)
2. Significant difference *** ($p<0.001$), NS= ($p>0.05$)
3. Calculated as nitrogen retained/ nitrogen absorbed x 100

Table 6.14. The effect of amino acid availability on excreta moisture, water intake and other parameters of laying hens

	Lysine availability[1] (g/kg lysine)							
	671.6	719.7	767.7	815.8	863.9	912.0	SEM[2]	Significance[3]
Excreta moisture (g/kg)	707.5	691.7	722.8	719.2	728.8	737.9	24.30	***
Water intake (g/b/day)	193.6	219.4	224.1	242.8	243.3	254.1	25.45	***
Food intake (g/b/day)	112.7	126.3	134.4	132.7	132.2	126.4	18.22	NS
Water: feed intake (g/g)	1.77	1.73	1.70	1.84	1.85	2.10	0.253	*
Total excreta (g/b/day)	106.5	118.5	127.3	130.0	131.2	135.9	15.63	***
Water excreted (g/b/day)	75.5	82.2	92.1	93.9	95.6	100.3	12.57	***
Dry matter excreted (g/b/day)	30.90	36.33	35.18	36.10	35.51	35.65	4.253	NS
Excreta score	3.13	3.38	3.13	3.13	3.50	3.13	0.784	NS

1. Lysine availability determined by True available amino acid (TAAA) assay. Availabilities of all other amino acids were also determined and reduced with heat treatment
2. df=39 (all parameters)
3. Significance level of the slope (b) where y=a+bx *** ($p<0.001$), ** ($p<0.01$), * ($p<0.05$), NS ($p>0.05$)

Table 6.15. The effect of amino acid availability on nitrogen metabolism of laying hens

	Lysine availability[1] (g/kg lysine)							
	671.6	719.7	767.7	815.8	863.9	912.0	SEM[2]	Significance[3]
Nitrogen intake (g/b/day)	3.20	3.46	3.45	3.49	3.48	3.49	0.486	NS
Nitrogen absorbed[4] (g/b/day)	1.74	2.06	2.13	2.21	2.47	2.23	0.433	*
Nitrogen excreted[4] (g/b/day)	1.93	2.05	1.97	2.00	1.96	1.93	0.275	NS
Nitrogen retention[4] (g/b/day)	1.26	1.42	1.49	1.47	1.52	1.59	0.419	*
Efficiency of N utilisation[5] (%)	70.00	68.50	68.10	66.30	69.40	63.10	8.060	NS
Uric acid excreted (g/b/day)	1.44	1.96	1.94	2.18	2.27	2.46	0.375	***
Uric acid nitrogen (g/b/day)	0.48	0.65	0.64	0.72	0.75	0.81	0.124	***
Faecal nitrogen[4] (g/b/day)	1.46	1.40	1.33	1.28	1.21	1.12	0.212	*
Uric N/ total N (%)	25.00	31.75	32.40	35.75	38.28	41.96	4.345	***
Serum uric acid (umol/l)	460.0	480.0	500.0	500.0	520.0	620.0	81.000	***

1. Lysine availability determined by True available amino acid (TAAA) assay. Availabilities of all other amino acids were also determined and were reduced with heat treatment
2. df=39 (all parameterss)
3. Significance level of the slope (b) where y=a+bx *** ($p<0.001$), ** ($p<0.01$), * ($p<0.05$), NS ($p>0.05$)
4. Apparent values
5. Calculated as nitrogen retained/nitrogen absorbed x 100

There were correlations ($p<0.05$) between the excreta moisture of the hens and the concentration of uric acid nitrogen ($r = 0.37$) in excreta, the ratio of uric acid nitrogen to total nitrogen in the excreta ($r = 0.32$), concentrations of uric acid in blood serum ($r = 0.34$) and faecal nitrogen excretion ($r = 0.31$) ($p<0.05$).

There were no effects ($p>0.05$) of reduced amino acid availabilities on ileal, caecal or colonic weight (tissue + contents), caecal digesta wet weight or caecal tissue wet weights (Table 6.16.).

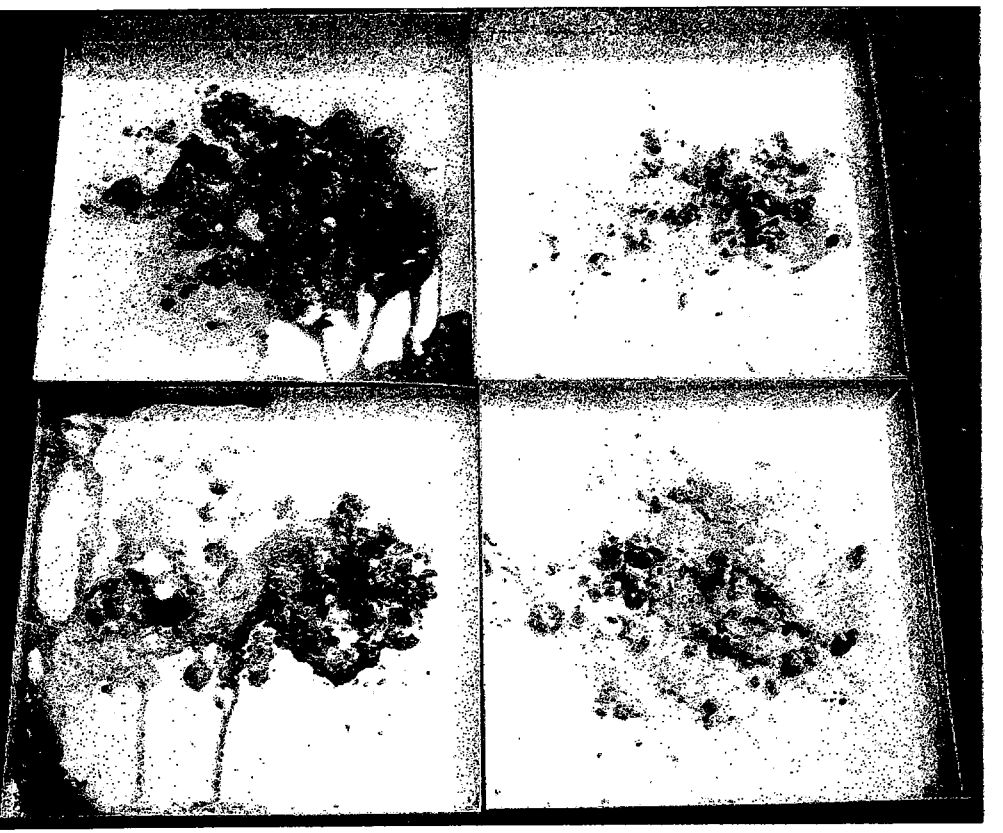


Figure 6.5. A comparison of excreta characteristics of birds fed de-hulled soybean meal, either heat treatet (left), or non heat treated (right)

Table 6.16. Effect of feeding diets with varying levels of amino acid availability on some ileal, caecal and colonic parameters of laying hens

	Diet			
Parameter	Non heat treated de-hulled soya bean meal	Heat treated de-hulled soya bean meal	SEM[1]	Significance
Total caecal weight (g/100 g body weight)	0.79	0.81	0.145	NS
Caecal digesta (g/100 g body weight)	0.37	0.36	0.156	NS
Caecal tissue wet weight (g/100 g body weight)	0.44	0.42	0.088	NS
Total ileal weight (g/100 g body weight)	5.00	5.27	1.092	NS
Total colonic weight (g/100 g body weight)	0.43	0.61	0.153	NS

1. Error df = 6 (all parameters)
2. Significant difference NS= ($p>0.05$)

6.4. DISCUSSION

6.4.1. Dietary crude protein concentration Increased dietary crude protein concentration produced a linear increase ($p<0.001$) in the moisture contents of excreta of hens, which agrees with the observations of both Lopez (1994) and Ogungi *et al* (1983) in broiler breeder hens and broiler breeder males respectively (Table 6.17.). However James and Wheeler (1949), Wheeler and James (1950), Patrick (1955) and Marks and Pesti (1984) all concerned with immature birds (Table 6.17.) reported there to be no effect of dietary crude protein concentration on excreta moisture. It could therefore be suggested that the effect of increased dietary crude protein concentration on excreta moisture differs between mature and immature birds, perhaps due to differences in nitrogen retention and excretion. Increased excreta moisture and water intakes, with increased dietary crude protein concentration, have been related to the need for additional water to excrete excess nitrogen as uric acid (e.g. Leeson *et al*, 1995) as the concentrating capacity of the kidneys are limited by the level of nitrogenous products for excretion (Skadhauge, 1973).

The quantitative estimate for excreta moisture derived from this experiment was greater than the estimate described by Lopez (1994) in broiler breeder hens, and that of Ogungi *et al* (1983) in broiler breeder males (Table 6.17.). The latter was obtained with a restricted feeding programme which would lead to anomalous data, and therefore was not examined further. A comparison of estimates for the effect of increases in crude protein concentration on nitrogen excretion showed the increase in this experiment to be greater ($p<0.05$) than in the work of Lopez (1994). This difference in nitrogen excretion may explain the difference in the two estimates of excreta moisture.

The source of additional dietary crude protein could effect nitrogen excretion. Patrick (1955) and Wheeler and James (1950) noted different effects of increased crude protein concentrations on

water intake and excreta moisture with different crude protein sources. Protein sources differ in their digestibilities and balance of amino acids. Higher crude protein digestibilities would increase nitrogen retention and excretion of uric acid, and give smaller quantities of dry matter for excretion. Previous workers used soya bean meal in replacement for maize, to alter crude protein concentration in the diets, whereas the present work used egg protein. Egg protein is easily digestible and completely used metabolically (Larbier and Leqlerque, 1994) whereas protein sources associated with carbohydrate molecules such as soya bean meal are less well digested. This was supported by Glista and Scott (1949) who found the level of faecal dry matter to increase as the level of soya bean meal increased in diets despite no increase in crude protein concentration.

Previous experiments which have examined the increase in excreta moisture and water intake with increased dietary crude protein concentration, have not examined crude protein concentrations in excess of requirements (Table 6.17.). At high nitrogen intakes, where maximal rates of protein synthesis have been met, nitrogen excretion has been shown to increase relative to the linear increase in nitrogen retention, lowering efficiency of nitrogen utilisation (Carr *et al,* 1977). The efficiency of nitrogen utilisation declined in this experiment with increased crude protein concentration, and the basal diets met all nutrient requirements of the laying hen. This may also therefore partly explain the higher estimates in this experiment than in previous experiments.

Linear increases ($p<0.001$) in nitrogen retention, blood serum uric acid concentration, total nitrogen, uric acid nitrogen and the proportion of nitrogen excreted as uric acid nitrogen with increased dietary crude protein concentration, were found in this experiment. These were consistent with the observations of Carr *et al* (1977) who have shown that increased nitrogen intakes increase both nitrogen retention and excretion, and results of several other studies which have shown that increases in crude protein intake increase both total nitrogen and uric acid

Table 6.17. A comparison of experimentally determined and previously published estimates for excreta moisture and water intakes with increasing concentrations of dietary crude protein

Author	Bird	Water intake	Excreta moisture	Source of additional crude protein	Range of treatments fed (g/k
Current	Laying hens	Y=188.9+0.746X	Y=410.85+1.32X	Dried whole egg	140-250
Lopez (1994)	Broiler breeder hens	.....	Y=643.5+0.95X	Soyabean meal +synthetic amino acids	90-150
Ogungi *et al* (1983)	Broiler breeder males	No effect	Y=705.0+0.75X	Soyabean meal	120-180
Marks and Pesti (1984)	Day old male chicks	.....	No effect	Soyabean meal	170-260
Patrick (1955)	Growing chicks	No effect	No effect	-Soyabean meal	210-270
		Decreased	No effect	-Casein	
		No effect	No effect	-Fishmeal	
James and Wheeler (1949)	Growing chicks	Y=56.83+0.295X	No effect	Soyabean meal	150-250
Wheeler and James (1950)	Growing chicks	Y=108.33+0.290X	-No effect	-Soyabean meal	150-250
		Increased	-No effect	-Fish meal	
		Increased	-No effect	-Meat scrap	

..... -no data presented
No effect- quantitative data provided but no significant effect recorded

excretion (Tasaki and Okumura, 1964; Featherstone and Scholz, 1968; Teekell *et al,* 1968; Mc Nab *et al,* 1972, 1973; Fernandez-Figares *et al,* 1995) and increase plasma uric acid concentrations (Okumura and Tasaki, 1968; Featherstone, 1969). Correlations ($p<0.001$) were observed in this experiment between the excreta moisture of the laying hens and these parameters. This is in line with data of Ward *et al* (1975) who have shown increased urine flow rates, and rates of uric acid nitrogen excretion with increased dietary crude protein in cockerels, and Dicker (1949) who found that plasma protein concentration as well as glomerular filtration rate were correlated with the protein concentration of the diet, and that within certain limits, glomerular filtration rate was correlated with urine flow. There were however no correlations ($p>0.05$) between excreta moisture and faecal nitrogen excretion. These results therefore strongly support previous suggestions (Patrick and Ferrise, 1962; Leeson *et al* 1995) that increased uric acid nitrogen excretion is a factor affecting excreta moisture with increased concentrations of dietary crude protein.

Confusion exists in previous work where the effect of crude protein concentrations on excreta moisture and water intakes were examined by replacement of maize for soya bean meal as soya bean meals have high electrolyte concentrations, which could effect mineral intakes, and anion cation balance (Mongin, 1989). Increased dietary mineral concentrations are known to increase excreta moisture (Smith *et al,* 1997 a). The estimates for the increases in excreta moisture and water intakes, with dietary crude protein concentrations could therefore have been confounded by un-intentional variation in electrolyte supplies in the compared diets. There was no evidence of any variation in electrolyte concentrations in the diets of this experiment. This suggests that there is an effect of dietary crude protein concentration *per se* on excreta moisture and water intakes, when in a readily available form.

The crude protein concentration of dietary components is subject to considerable variation.

Through more precise control of the quantity of components rich in crude protein, and a more widespread use of synthetic amino acids to lower crude protein concentrations in ration formulations, a considerable proportion of variation in excreta moisture could be controlled.

6.4.2. Dietary lysine and methionine concentration No previous estimates for the effects of dietary lysine and methionine concentrations on water intakes and moisture contents of excreta were found. Experiment 7 indicated that increased dietary lysine concentration produced a linear increase ($p<0.001$) in both water intake and the moisture contents of excreta, and that the effects of dietary lysine and protein concentration were additive. These observations were consistent with the subjective observations of Brooks and Carpenter (1990) in pigs, that the more the amino acid supply deviated from ideal, the greater the demand for water intake. The additive effects of crude protein and lysine concentration were consistent with observations on amino acid excess of Harper *et al,* (1970), that if amino acid concentration is increased in direct proportion to protein concentration of the diet, the additional protein will have no effect on the response to the increased amino acid concentration.

However, experiment 8 indicated that, unlike an increase in dietary lysine concentration, increased methionine concentration had no ($p>0.05$) effects on the moisture content of the excreta and decreased ($p<0.001$) water intakes. The differences between methionine and lysine concentrations are consistent with the view of Harper *et al* (1970) that individual amino acids, when in excess, do not have common features. There were also no methionine concentration x lysine concentration interactions which again may suggest that individual amino acids have independent effects.

Individual amino acids provided in excess, in relation to those which are limiting, are poorly retained (Larbier and Leqlerque, 1994). Bedford and Summers (1985) state that an excess of either non - essential or essential amino acids will increase amino acid catabolism, and Fernandez -

Figares *et al* (1995) have shown that lowered protein quality may increase uric acid nitrogen excretion. An excess of either methionine or lysine would therefore be expected to stimulate relevant degradative pathways, increasing uric acid nitrogen excretion, as for an excess of crude protein. Significant linear decreases in nitrogen retention, and a linear increase in total nitrogen excreted, uric acid nitrogen excreted and serum uric acid concentrations, with increased dietary lysine concentrations, are consistent with this. Significant correlations between the excreta moisture of the hens and the concentrations of uric acid nitrogen, and ratio of uric acid nitrogen to total nitrogen in excreta, and concentration of uric acid in blood serum, suggest that, as with increased crude protein concentration, increased excretion of uric acid nitrogen may be the factor causing an increase in excreta moisture with increased dietary lysine concentration.

Although increased dietary methionine concentration also reduced nitrogen retention ($p<0.001$) it gave linear decreases in nitrogen excretion ($p<0.05$), serum uric acid concentration, uric acid nitrogen excretion, and in the ratio of uric acid nitrogen to total nitrogen excreted ($p<0.001$). This difference in both the nitrogen metabolism, and the effect on the excreta moisture and water intakes from dietary lysine, could lie in the difference in the effects of each on feed intake. The effects of imbalances appear to be mediated primarily by changes in food intake (Sanahuja and Harper, 1963; Rogers and Leung, 1973). Decreases in feed intakes were observed with both increased dietary lysine, and dietary methionine concentrations. However, reductions in feed intake with dietary methionine were more than two fold greater than for an equivalent increase in dietary lysine concentration, consistent with the low dietary concentration of methionine required to produce toxicity (Larbier and Leqlerque, 1994). The greater reduction in feed intakes gave large reductions in nitrogen intakes and nitrogen absorption, and may have caused non - essential amino acids to become limiting. Limiting concentrations of non - essential amino acids may increase transamination of methionine in preference to catabolism, reducing the water requirement for the excretion of excess nitrogen.

A large proportion of the variation in water intakes in the hens with increasing dietary methionine concentrations, were explained by feed intake. Under *ad libitum* conditions water intake is related directly to food eaten (Patrick and Ferrise, 1962; Ibarbia, 1968). Any reduction in water intake with reduced feed intake with dietary lysine, would be counteracted by the requirement for a greater water intake to excrete excess uric acid. In contrast with a dietary methionine excess, water intakes decline in line with feed intakes. There were therefore no effects on water to feed intake ratios and no effect on the excreta moisture of the hens. It therefore appears that the expected response in excreta moisture, due to variation in nitrogen metabolism with increased methionine concentration, may be hidden by the more profound effect of decreased feed intake.

Synthetic free lysine and methionine may both be absorbed shortly after consumption and may be utilised less efficiently than the amino acids of dietary proteins which are released and absorbed together with other nutrients over a longer period. The observed effects of feeding graded concentrations of free amino acids may be more severe than when feeding natural ingredients as free amino acids may be absorbed before other amino acids are available for tissue synthesis, and consequently will be partially degraded and excreted.

6.4.3. Amino acid availability Heat treatment of the de-hulled soyabean meal reduced the availability of the essential amino acids at least by 25 %. Current values for the non - heat treated form were consistent with those found for de-hulled soya bean meal by Yamazaki (1983) using the TME procedure of Sibbald (1979) but lower than those of Likuski and Dorrell (1978) and Sibbald (1979) with 24 h collection periods. Heat treatment lowered the availability of amino acids to a similar level to observed by Hayes *et al* (1983) in fish meal, when exposed to similar conditions.

No previous estimates for the effect of reduced amino acid availability on moisture content of

excreta, or water intake in laying hens were found. Reduced amino acid availabilities of the crude protein source in the diets of the current experiment gave linear decreases ($p<0.001$) in both the water intakes, and the moisture contents of excreta of laying hens.

The percentage decreases in availability of each amino acid measured were similar within the crude protein source, and therefore the balance of amino acids available at the site of protein synthesis would have remained similar. The effect of reduced amino acid availabilities were therefore consistent with a reduction in crude protein, both in terms of nitrogen metabolism of the hens, and the effects on excreta moisture and water intake. However susceptibility of amino acids to heat treatment varies. All amino acids were affected in this experiment, due to the severe heat treatment imposed. Less severe conditions, such as those found in practice, may affect only those amino acids such as lysine, with characteristics which render them particularly susceptible to heat. This would disturb the balance of amino acids available at the site of protein synthesis, and therefore may produce a different estimate of excreta moisture and water intake to the current observations.

There was a significant negative correlation between faecal nitrogen excretion and excreta moisture of the laying hens. There were therefore no effects of increased concentrations of amino acids reaching the hindgut on excreta moisture in this experiment. However, there were significant correlations between the excreta moisture of the laying hens and the concentrations of uric acid nitrogen in excreta, the proportion of nitrogen excreted as uric acid nitrogen, and the concentration of uric acid in serum. These observations were consistent with previous observations in this series of experiments that suggest uric acid nitrogen excretion may be a factor effecting excreta moisture.

There were no effects of the diets on the excreta score after feeding for eight days. However figure 6.5. shows that when de-hulled soya bean meal of lowered amino acid availability was force fed to adult cockerels in a TAAA assay, a highly viscous excreta was produced, with copious amounts

of free water. Similar, but transient observations were made in laying hens fed diets containing higher concentrations of the heat treated de-hulled soya bean meal. These observation were consistent with subjective observations of Jordan (1990), that reduced amino acid availabilities may cause sticky droppings. Amino acids which are not absorbed in the small intestine may pass to the caeca and colon where they become subject to fermentation by the microbial flora (Austic, 1983). Although the microflora of the mature bird is both active and stable (Johnson, 1987) high concentrations of unabsorbed amino acids may give a transient increase in the microflora, which could effect excreta quality, both through an increased proportion of osmotically active fermentation products, and an increased rate of evacuation of highly viscous enteric excreta. Previously published work has linked other unabsorbed dietary components to wet and sticky droppings (e.g. Leegwater *et al,* 1974; Longstaff *et al*, 1988; Choct and Annison, 1992 b). However none of these reported the effect to be transient.

There were no effects of the reduced amino acid availabilities on ileal, caecal or colonic wet weights, caecal contents or caecal tissue weight after eight days. This may suggest that there were no differences in the levels of fermentation taking place between diets after eight days as longer and heavier caeca have been related to increased levels of fermentation (Longstaff *et al,* 1988).

Williams (1995) stated that nitrogen excretion can be minimised by accurately matching the composition of dietary nitrogen with the animal requirement, and achieving a minimal level of total dietary nitrogen of an ideal composition. By meeting these requirements thereby minimising nitrogen excretion, excreta moisture may also be reduced.

6.5. CONCLUSIONS

In conclusion, increasing the concentration of a well balanced, available dietary crude protein source, independent of electrolyte concentration, in laying hen diets produced a linear increase in the moisture contents of excreta. A comparison of current and previously published estimates suggests the estimate may be influenced by the digestibility of the crude protein source. An experiment examining the effect of variation in amino acid availability on excreta moisture, verified this observation, showing linear increases in excreta moisture, with increased availability. Two further experiments showed that the response in excreta moisture of laying hens to increased dietary concentrations of individual amino acids at constant crude protein concentration to vary depending on the individual amino acidswhen in excess of requirement. Increases in the ratio of lysine to crude protein concentration produced a linear increase in the excreta moisture, which was additive with crude protein concentration effects. In contrast, an excess of dietary methionine had no effect on excreta moisture, and lysine effects were not altered by the methionine concentration. Strong correlations between the uric acid nitrogen content of excreta, and excreta moisture, suggest variation in uric acid excretion may be a causative factor of variation in excreta moisture, with variation in nitrogen intake. There may be an economic benefit to laying flocks of accurately matching the composition of dietary nitrogen with the animals requirement, to reduce excreta moisture.

CHAPTER 7.

THE EFFECT OF DIETARY CONCENTRATIONS OF VARIOUS CARBOHYDRATE SOURCES ON THE EXCRETA MOISTURE AND WATER INTAKES OF LAYING HENS

7.1. INTRODUCTION

The carbohydrate fraction is quantitatively the most important component of the diet of the laying bird, and comprises free sugars, starch, cellulose and non - starch polysaccharides. Starch represents about 600-700 g/kg of most cereals, a larger proportion of many roots and tubers, and is a major component of many legumes, such as peas and beans (El Faki *et al,* 1984).

A widely held assumption is that starch is completely hydrolysed and absorbed in the small intestine(Dahlquist and Borgstrom, 1961). It is now known that the extent of starch digestion in the small intestine is variable, and that a substantial amount, depending on physical form, escapes digestion in the small intestine and may enter the colon and be fermented. When first identified (Englyst *et al,* 1992) this fraction consisted largely of retrograded amylose. Since then it has been shown that a variety of types of starch exist and that the rate and extent of digestion in the small intestine varies for a number of reasons.

Englyst *et al* (1992) have shown that, compared to foodstuffs such as wheat flour, leguminous foodstuffs are relatively high in resistant starch and Yutste *et al* (1991) have shown that starch digestion in birds is poorer in feedstuffs known to cause high excreta moisture. A number of workers have made subjective observations on the effects of graded concentrations of dietary ingredients known to contain raised concentrations of resistant starch such as tapioca, soyabean meal and other legumes on excreta moisture (Cooke and Raine, 1986; Pattison, 1989). However these workers did not examine the effect of resistant starch *per se,* did not use the laying hen as a model and did not provide quantitative data.

Cereal grains are also important sources of dietary fibre (Wisker *et al,* 1985). The fibre fraction comprises cellulose and non - starch polysaccharides. The two major non - starch polysaccharide

components of cereal fibre are the pentosans (arabinoxylans) which are polymers of β-1-4 linked D-xylose residues of variable length, with single L-arabinosyl furanosyl residues substituted at the O2 and O3 position of the xylose, and the 1-3 1-4 mixed linked β-glucans (Henry, 1986) which are polymers of β-D-glucopyranose units joined by either 1-3 (30%) or 1-4 (70%) β-glycosidic bonds. Both contain highly soluble viscous fractions, and are specifically associated with the primary cell walls of starch endosperm and thick walled aleurone (Fincher and Stone, 1981).

There is little data that quantitatively describes the increase in excreta moisture to different dietary concentrations of resistant starch *per se* in the laying hen. Although considerable data exists to link non - starch polysaccharides to increased excreta moisture, few published experiments have used the laying hen as a model and few have derived any quantitative estimates of the effects of concentrations of non - starch polysaccharide on excreta moisture. Numerous workers (e.g. Herstadt, 1987) have suggested that the detrimental effects of non - starch polysaccharide on layers are smaller than in broiler chickens. Two separate experiments were therefore carried out in this project, to quantitatively measure the effect of increasing dietary concentrations of resistant starch *per se* on water intake and excreta moisture of laying hens, and secondly, to measure the effect of increasing dietary concentrations of soluble non - starch polysaccharide, by a comparison of diets containing one of four concentrations of three cereal types known to differ in their soluble non - starch polysaccharide concentration, and two concentrations of wheat bran to test for any soluble x insoluble non - starch polysaccharide interactions on the excreta moisture, and water intake, of laying hens.

7.2. MATERIALS AND METHODS

7.2.1. Experiment 10 The objective of this experiment was to quantitatively measure the effect of varying dietary concentrations of resistant starch, as a proportion of total dietary starch, on the excreta moisture and water intakes of laying hens.

Forty eight, 38-week-old ISA Brown laying hens were caged in individual wire floored cages (50 x 45 x 45 cm) arranged in 4 tiers, within an environmentally controlled room. Each cage had an individual feed hopper, water trough and excreta collection tray. The birds were maintained under a 14L:10D lighting regime at 24 ±1 °C and 80 ±5% relative humidity.

Diets were semi-synthetic laying hen rations (11.40 MJ/kg of ME and 166.0 g/kg of crude protein) identical in nutrient composition, except in the composition of the starch fraction (Table 7.1.). The diets were formulated to contain 6 concentrations of resistant starch (0, 50, 100, 150, 200 and 250 g/kg of total starch) by replacement of a portion of readily digestible (100 g/ 100 g dry matter RDS) starch (soluble starch, Sigma S4126) with a resistant (6, 19 and 75 g/100 g dry matter of RDS, SDS and RS respectively), starch (potato starch, Sigma S4251) (Table 7.2.). The metabolisable energy concentration of potato starch was lower than for soluble starch (10.5 MJ of ME/ kg and 15 MJ/ kg respectively[1]). To maintain equal metabolisable energies for each diet, total starch values were allowed to increase at the expense of washed sand, as concentrations of potato starch increased (Table 7.2.).

1. Prior to formulation of experimental diets 24 Isabrown cockerels were used, in an attempt to determine the true metabolisable energy (TME_n) of potato starch, and corn starch samples using the modified procedure described by Mc Nab and Blair (1988). Ten birds were fed each starch type, and two were used to determine endogenous energy losses. When tube feeding birds considerable problems were presented due to the powder like consistency of starches, causing both compaction within the tube and loss to the environment. The procedure was repeated with starches in a 50 ml water based suspension. Considerable regurgitation resulted. It was decided to forfeit this procedure and to estimate metabolisable energy content of potato starch, assuming both to contain 15 MJ of GE per kg but allowing for the reduced digestibility of potato starch, suggested by Yutse *et al*, (1991) to be 70% in the adult cockerel, in calculation of ME.

Table 7.1. Composition (g/kg) and analysis of basal diets for experiments 10 and 11

	Experiment	
Component	10	11
Wheat	268.0	.....
Barley	60.0	.....
Maize	.....	500.0[1]
Maize gluten meal	70.0	70.0
De-hulled soya bean meal	110.0	160.0
Fish meal	21.5	.....
Meat and bone meal	34.5	.....
Lysine HCl	4.5	6.0
Methionine	1.5	3.0
Tryptophan	.....	0.5
Soya oil	67.3	56.0
Limestone	71.0	86.5
Dicalcium phosphate	6.0	16.5
Sand[2]	152.0	82.9
Soluble starch (RDS)[3]	121.0	.....
Sodium chloride	0.5	1.5
Potassium carbonate	.....	1.6
Magnesium chloride	.....	3.0
Vitamin mineral premix[4]	12.5	12.5
Analysis (calculated)		
Crude protein	166.0	170.1
Starch	391.9	321.6
Lysine	10.4	11.6
Methionine	5.2	6.4
Potassium	4.4	6.8
Sodium	1.6	1.7
Calcium	38.9	42.0
Phosphorus	4.1	4.2
Chloride	0.8	1.2
ME (MJ of ME/kg)	11.4	11.9

1. Maize substituted for wheat and or barley in formulation of the experimental diets for experiment 11
2. Washed builders sand used in replacement for total starch in experiment 10 and wheat bran in experiment 11 as indicated in tables 7.2 and 7.4 respectivly
3. Soluble starch fed at six different concentrations in experimental diets of experiment 10 in replacement for potato starch
4. Comprised ash (890 g/kg), calcium (250 g/kg), methionine (80 g/kg), sodium (88 g/kg), copper (cupric sulphate 400 mg/kg), vitamin A (480000 i.u./kg), vitamin E alpha tocopherol acetate (480 i.u./kg), vitamin D3 (240000 i.u./kg)

Table 7.2. Composition and experimentally determined analysis of dietary starch in experimental diets of experiment 10

	Dietary resistant starch (g/kg total starch)							
Component	0.0	50.0	100.0	150.0	200.0	250.0	SEM[1]	Significance
Soluble starch	121.0	116.0	112.5	107.0	101.0	95.0	.....	.....
Potato starch	0.0	16.8	34.0	54.0	78.0	100.0	.....	.....
Sand[2]	152.0	94.0	126.5	111.5	94.0	78.0	.....	.....
Basal starch	195.9	195.9	195.9	195.9	195.9	195.9	.....	.....
Total starch[3]	316.9	329.2	342.4	357.4	379.4	390.9	.....	.....
Analysis[4]								
Resistant starch (g/kg total starch)	20.99±0.764	65.40±1.131	132.89±0.601	177.35±1.767	224.45±7.071	235.75±2.616	2.461	***
Slowly digestible starch (g/kg total starch)	391.00±5.656	399.45±0.777	464.35±3.599	461.65±2.616	494.00±2.828	509.80±0.735	2.254	***
Readily digestible starch (g/kg total starch)	587.90±4.893	534.80±0.636	402.50±2.757	360.80±1.131	281.50±10.394	254.60±3.563	3.580	***
Total starch (g/kg diet)	327.87±1.513	305.27±3.302	327.45±1.626	334.49±2.192	351.77±2.666	368.55±1.223	1.561	***
ME (MJ of ME/kg) (calculated)	11.39	11.39	11.39	11.39	11.39	11.39	.....	.....

1. Error df = 6 (all parameters)
2. Washed sand used as a filler in replacement for increasing levels of total starch
3. Total starch levels increased with addition of resistant starch to maintain constant metabolisable energy
4. Analysis of diets performed in duplicate using an Englyst starch kit (Novo Biolabs) (Appendix 8) according to the classification of Englyst and Cummings (1987)

Starches other than resistant starch are digested almost completely in the small intestine, and therefore are unlikely to reach the large intestine to affect water balance (Longstaff and Mc Nab, 1987; Rodriguez *et al*, 1987). Any observed effects on excreta moisture could therefore be attributed to resistant starch. Macro - mineral concentrations were maintained constant by replacement of washed sand with inorganic mineral salts. Each bird had *ad libitum* access to one of the six diets and water throughout the 8 day feeding period. Water intakes and feed intakes were measured and all excreta collected for each 24 h period within the final two days of the feeding period. Excreta were collected in open trays and the moisture contents of the excreta were determined by drying at 60 °C in a forced air oven. Data was then corrected for loss of moisture to the environment, using the correction factor obtained in experiment 1. Water and feed intakes were determined by weighing the appropriate trough at the beggining and the end of the collection period. Water intakes were adjusted for loss of water vapour from the drinkers. Only data from egg laying days was used in calculation of treatment means, to reduce variation as a result of a response in water balance to egg formation (Wood- Gush and Horne, 1970).

The experiment was designed as a randomised block analysis of variance with cage tier level as a blocking factor. Treatment sums of squares were partitioned into a set of orthogonal linear and quadratic polynomial regression components, using the GENSTAT statistical package (Lawes Agricultural trust, 1984). Multiple regression analyses, with water intake and feed intake as independent variables, and excreta moisture as a dependant variable, were also carried out.

7.2.2. Experiment 11 The first objective of this experiment was to quantitatively investigate the changes in excreta moisture and water intake with differing concentrations of dietary soluble non - starch polysaccharides, by a comparison of diets containing four concentrations of three different cereal sources, (wheat, barley and maize), known to differ in their soluble non - starch polysaccharide content, and a second objective was to examine for any soluble x insoluble non -

starch polysaccharide concentration interactions by a comparison of the diets, when fed with one of two concentrations of wheat bran. A third objective was to measure the effect of the diets on the viscosity, moisture contents and wet weights of caecal and ileal digesta of laying hens.

Ninety six, 42-week-old ISA Brown laying hens were housed in the same cages and given an identical lighting regime, temperature and relative humidity as described in experiment 10.

Samples of a 1993 harvest Australian wheat (believed to contain high concentrations of non - starch polysaccharide), a commercially grown de - hulled barley (pearled barley), dried rolled maize and wheat bran (Table 7.3.) were used to formulate twenty four experimental diets that were identical in nutrient composition, except for their concentration of non - starch polysaccharide. Each was a typical laying hen ration, (11.9 MJ/kg of ME and 170 g/kg of crude protein), and contained 500 g/kg as cereal (Table 7.1.). Each diet contained a combination of five concentrations (0, 125, 250, 375 or 500 g/kg) of wheat, barley and maize and one of two concentrations of wheat bran, (0 or 100 g/kg), at expense of maize gluten, maize starch, soyabean oil and washed sand, to maintain constant crude protein and metabolisable energy levels (Table 7.4.). Macro - mineral concentrations were maintained constant by replacement of washed sand with inorganic mineral salts. Each bird had *ad libitum* access to one of the twenty four diets and water throughout the 8 day feeding period.

Water intakes and feed intakes were measured, and all excreta collected for each 24 h period of the final two days of the feeding period. The moisture contents of the excreta were determined by drying at 60 °C in a forced air oven. Data was then corrected for loss of moisture to the environment, using the correction factor obtained in experiment 1. Water and feed intakes were determined by weighing the appropriate trough at the beggining and the end of the collection period. Water intakes were adjusted for loss of water vapour from the drinkers. Only on egg laying

Table 7.3. Composition (g/kg DM) of cereals used in formulation of experimental diets for experiment 11

Component	Crude fibre	Neutral detergent fibre	Nitrogen
Wheat	30.33±0.410	101.04±3.84	2.88 ±0.040
Barley	8.80±0.355	43.28±7.34	1.74 ±0.017
Maize	25.73±0.686	118.18±1.695	1.59±0.032
Wheat Bran	102.17±0.305	430.36±6.69	2.50±0.031

Table 7.4. Composition (g/kg) and analysis of experimental diets of experiment 11

	Composition of experimental rations (g/kg of diet)									
	Ingredient								Chemical analysis	
	Maize	Barley	Wheat	Wheat bran	Maize gluten	Sand	Soya bean oil	Maize starch	Crude fibre	Neutral detergent fibre
Diet										
1	500	.....	.....	.....	70.0	82.9	56.0	.....	17.62±3.108	61.50±1.861
2	500	.....	.....	100	44.0	19.0	52.0	.....	28.07±4.370	110.90±2.961
3	375	125	.....	.....	66.2	79.6	59.0	4.0	16.47±1.104	70.34±2.620
4	375	125	.....	100	40.2	19.7	55.0	.....	25.08±2.009	97.86±0.741
5	375	.....	125	.....	67.7	79.9	59.7	4.5	20.99±0.526	62.54±2.736
6	375	.....	125	100	38.0	21.6	55.0	1.0	30.52±0.488	104.80±1.150
7	250	250	.....	.....	62.5	71.9	62.0	12.5	13.95±1.195	66.48±2.544
8	250	250	.....	100	36.5	13.0	57.7	8.0	26.48±0.094	94.60±2.639
9	250	.....	250	.....	61.5	71.9	63.5	13.0	17.85±0.568	67.71±8.860
10	250	.....	250	100	36.5	14.6	59.5	8.0	26.28±2.388	102.54±2.051
11	125	.....	375	.....	57.2	63.6	67.0	22.2	18.09±1.275	57.38±2.142
12	125	.....	375	100	31.5	5.7	62.7	18.0	25.20±2.510	92.12±1.534
13	125	375	.....	.....	58.7	64.4	65.0	21.0	10.10±0.298	49.23±0.075
14	125	375	.....	100	33.0	8.7	61.0	12.5	23.75±3.290	84.57±0.484
15	.....	500	.....	.....	54.7	55.9	67.5	32.0	15.09±4.964	47.88±7.070
16	.....	500	.....	100	28.7	.....	63.5	25.0	19.95±3.622	78.02±5.890
17	.....	375	125	.....	54.5	55.6	68.2	31.5	16.77±5.081	58.42±4.450
18	.....	375	125	100	28.0	.....	64.2	26.0	19.95±1.139	94.85±5.420
19	.....	250	250	.....	54.2	55.2	65.0	43.7	25.28±0.217	80.16±1.711
20	.....	250	250	100	27.5	14.0	65.0	25.2	26.39±0.375	86.95±2.969
21	.....	.....	500	.....	53.0	57.7	70.2	34.0	21.83±0.510	68.87±5.614
22	.....	.....	500	100	26.2	.....	66.2	26.0	33.11±0.267	109.73±7.155
23	.....	125	375	.....	53.5	54.1	69.7	32.5	20.74±0.036	64.55±3.019
24	.....	125	375	100	27.2	0.0	65.7	25.5	31.40±0.122	99.01±2.382

days was data used in calculation of treatment means.

On day eight excreta were scored on both collection days according to their consistency. The scoring system placed excreta into one of six divisions according to the consistency of the excreta from (1) pasty to (6) particulate. Mean values were determined for each diet. Twenty four birds (1 per treatment) were subsequently weighed and slaughtered using a 0.5 ml intravenous injection of sodium pentobarbitone, to avoid any disturbance in digesta distribution through contraction of intestinal musculature. Caeca (below the ileo - colonic - caecal junction) and ileum (between Meckel's diverticulum and the ileo - colonic - caecal junction) were removed and weighed. Digesta were removed from each, by manual compression, on to a pre - weighed container. Digesta and tissue were individually weighed. A 2 g sample of digesta was reserved for viscosity determination and the remainder dried at 60 °C in a forced air oven, to determine moisture content. Samples for viscosity determination were centrifuged at 12000 x g for five minutes to give a supernatant which was stored on ice before reading with a Brookfield model DV - II + viscometer (Brookfield Engineering Laboratories Inc, Stoughton, M.A. U.S.A.). Caecal digesta were very viscous, therefore, before centrifugation samples were first homogenised with 2 ml of deionised water.

The experiment was designed as a randomised block with a blocking factor of four cage tier levels. A multiple regression analysis with cereal source, bran concentration and cereal source x bran concentration interactions as independent variables, and measured parameters as dependent variables, was used to test for an effect of different cereal types, and for cereal source x bran concentration interactions. Analysis of variance was subsequently carried out to test for effects of wheat bran concentration.

All analysis was carried out using the GENSTAT statistical package (Lawes Agricultural Trust, 1984).

*7.2.3. **Feed analyses*** Samples of feeds for each experiment were ground to pass a 0.5 mm mesh screen and were subsequently analysed by standard proximate analysis (A.O.A.C., 1990).

Samples of soluble and potato starch, and each feed in experiment 10 were analysed *invitro* for rapidly available glucose (RAG), rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) by controlled enzymic hydrolysis and measurement of released glucose using the Englyst starch Kit (Novo Biolabs product no. 61000) according to the method described in Appendix 8. A modification of the procedure was used to determine the rate of digestion of potato starch and corn starch more precisely by measuring the cumulative amount of glucose released, following incubation with pancreatic amylase and amyloglucosidase after 15, 30, 45, 60, 120 and 240 minutes. Samples of wheat, barley, maize, wheat bran and finished feeds from experiment eleven were analysed for total water insoluble fibre by neutral detergent fibre estimation (Soest and Wine, 1967) using fibertec apparatus.

Sodium, calcium, phosphorus and potassium concentrations were determined with an atomic absorption spectrophotometer (Smith-Hieftje 1000, Thermo Jarrell Ash Corporation) following wet digestion (A.O.A.C., 1990). Inorganic phosphorus was determined using a colorimetric technique (MAFF, 1986), in which concentration of phosphorus in a trichloroacetic acid extract is determined spectrophotometrically as the yellow phospho - vanado - molybdate complex at 400 nM. Diets which failed to conform to the calculated analysis +/- 10% (Table 7.1) were re-mixed and re-analysed.

7.3. RESULTS

7.3.1. Experiment 10 (see Table 7.5.) The potato starch and soluble starch had differing *in vitro* rates of digestion (Figures 7.1. and 7.2. respectively). There were therefore linear increases in the concentrations (g/kg total starch) of slowly digestible starch, and resistant starch, in the six diets with increasing potato starch concentration ($p<0.001$) and a linear decrease ($p<0.001$) in readily digestible starch (Table 7.2.). There were no effects ($p>0.05$) of differing starch type or concentration on the sodium (1.79 ±0.079 g/kg), potassium (7.04 ±0.106 g/kg) or phosphorus (4.46 ±0.188 g/kg) concentrations of the diets.

There was no effect ($p>0.05$) of dietary resistant starch concentration on feed intakes, so increased dietary resistant starch concentration produced a linear increase ($p<0.001$) in resistant starch intake. Increased dietary resistant starch concentration increased water intakes ($p<0.05$), (Figure 7.3.) and water to feed intake ratios ($P<0.01$). There were also linear increases ($p<0.001$) in the weights of water excreted, weights of dry matter excreted, the moisture contents of the excreta (g/kg), (Figure 7.3.) and total outputs (fresh weights) of excreta.

7.3.2. Experiment 11 Cereal source effects Samples of wheat, barley, maize and wheat bran contained differing concentrations ($p<0.001$) of non - starch polysaccharide when determined as crude fibre or neutral detergent fibre (Table 7.3.). There were however no effects ($p>0.05$) of dietary wheat concentration on crude fibre or neutral detergent fibre concentrations of the diets, although increased barley concentration depressed neutral detergent fibre and crude fibre ($p<0.05$). There were no effects ($p>0.05$) of the differing cereal sources on the sodium (1.574 ±0.065 g/kg), potassium (4.428 ±0.098 g/kg) or phosphorus (3.808 ±0.120 g/kg) concentrations of the diets.

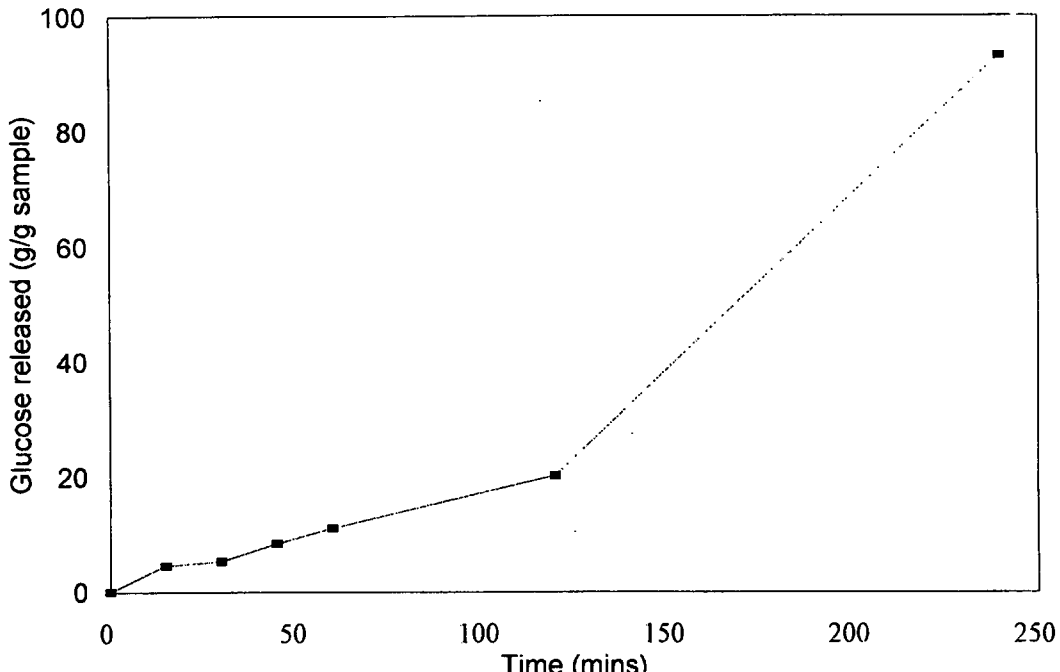


Figure 7.1.Invitro rate of digestion of potato starch

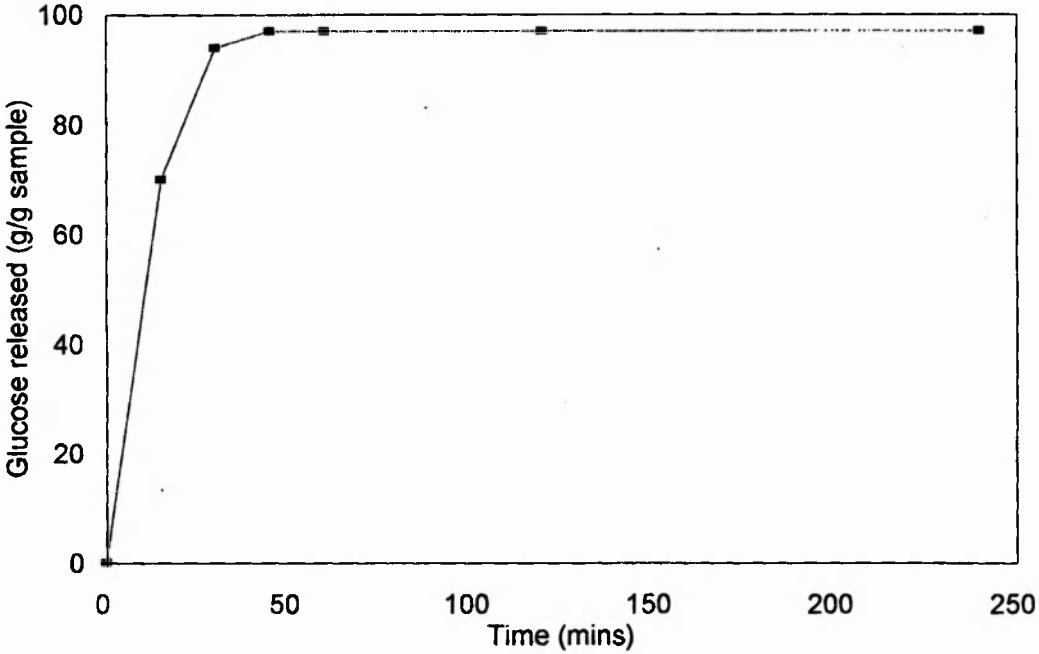


Figure 7.2. Invitro rate of digestion of soluble starch

Table 7.5. Effect of resistant starch intake on excreta moisture, water intakes and other parameters of laying hens

	Resistant Starch (g/kg of total starch)							
Parameter	0.00	50.00	100.00	150.00	200.00	250.00	SEM[1]	Significance[2]
RS intake (g/kg)	0.00	79.80	151.80	245.30	307.30	371.80	33.210	***
Food intake (g/b/day)	149.50	156.60	153.00	162.40	147.70	152.70	21.720	NS
Water intake (g/b/day)	269.60	295.40	302.70	316.30	310.60	320.00	37.240	*
Water: feed intake ratio (g/g)	1.81	1.91	1.97	1.95	2.17	2.11	0.275	**
Excreta moisture (g/kg)	676.30	698.50	706.00	709.20	717.10	718.30	26.470	***
Total excreta (g/b/day)	128.30	144.50	152.80	154.90	171.60	178.20	20.850	***
Water excreted (g/b/day)	83.90	101.00	108.10	109.80	123.20	127.70	15.460	***
Dry matter excreted (g/b/day)	44.40	43.50	44.80	45.10	48.30	50.50	7.340	**

1. Error df=39 (all parameters)
2.Significance level of the slope (b) where y=a+bx *** ($p<0.001$), ** ($p<0.01$), * ($p<0.05$), NS ($p>0.05$)

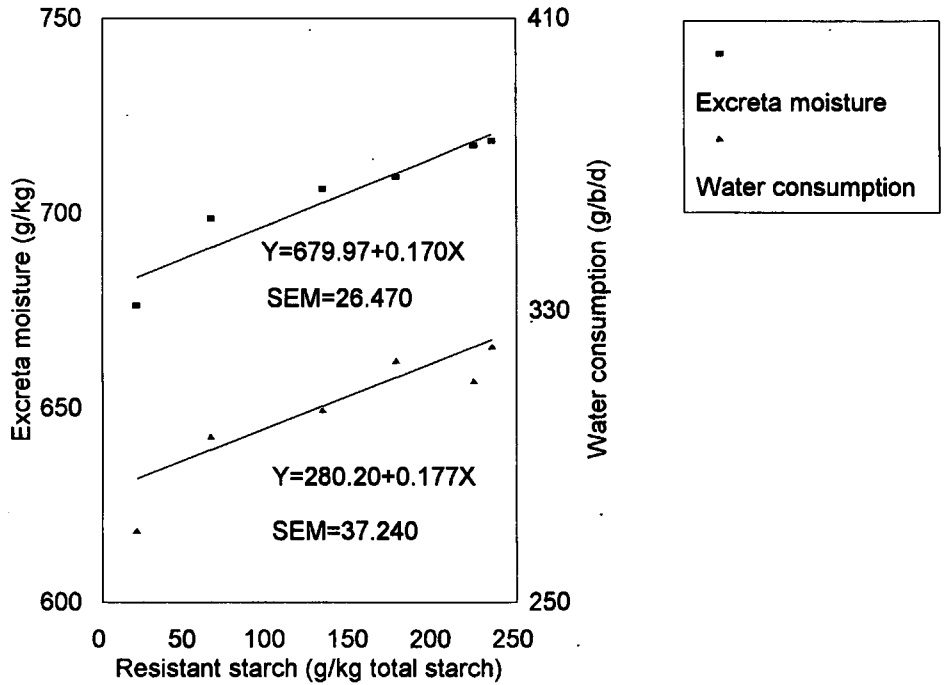


Figure 7.3. The effect of dietary resistant starch concentration (g/kg total starch) on excreta moisture and water intake of laying hens

Multiple regression analysis showed that there were no effects of differing cereal source ($p>0.05$) on feed intakes, water intakes, water to feed intake ratios, weights of water excreted, moisture contents of excreta or the consistency of excreta of the laying hens and that there were no cereal source x wheat bran concentration interactions ($p>0.05$) (Tables 7.6. and 7.7.). There were no effects ($p>0.05$) and no cereal source x wheat bran concentration interactions ($p>0.05$) for the wet weight, viscosity or moisture content of either the caecal or ileal digesta and no effects ($p>0.05$) on caecal or ileal tissue wet weight (Tables 7.8 and 7.9). For each parameter examined, multiple regression analysis showed that the majority of variation with diet was accounted for by dietary wheat bran concentration.

Wheat bran concentration effects Wheat bran contained high concentrations of crude fibre and neutral detergent fibre and therefore had a high soluble and insoluble non - starch polysaccharide concentration (Table 7.3.). Increased wheat bran concentrations of the diets therefore increased non - starch polysaccharide concentrations of the diets ($p<0.001$) as indicated by the crude fibre and neutral detergent fibre estimates (Table 7.10.) soluble and insoluble non - starch polysaccharide concentrations of the diets ($p<0.001$) (Table 7.10.). There was no effect ($p>0.05$) of dietary wheat bran concentration on feed intakes, so increased dietary wheat bran concentration increased non - starch polysaccharide intakes ($p<0.05$). Increased dietary wheat bran concentrations increased water intakes, weights of water excreted and excreta moisture ($p<0.01$), but had no effect ($p>0.05$) on the consistency of excreta (Table 7.10.).

There were positive linear relationships ($p<0.01$) between the concentration of non - starch polysaccharide measured as either crude fibre or neutral detergent fibre and excreta moisture ($r = 0.28$ and 0.27 respectively) and water intakes ($r = 0.24$ and 0.33 respectively) of the laying hens.

Increased wheat bran concentration raised ($p<0.05$) the wet weight, weights of water and the

Table 7.6. The effects of diets containing different concentrations of differing cereal sources on excreta moisture, water intake and other parameters of laying hens (low fibre diets)

	Diet												
	1	3	5	7	9	11	13	15	17	19	21	23	SEM[1]
Maize	500	375	375	250	250	125	125	.....	.....	.....	.....	.....	
Barley	.....	125	.....	250	.....	.....	375	500	375	250	.....	125	
Wheat	.....	.....	125	.....	250	375	.....	.....	125	250	500	375	
Excreta Moisture (g/kg)	762.0	762.9	709.2	703.5	728.5	735.2	713.5	776.4	773.8	764.1	727.2	757.0	**43.540**
Water intake (g/b/d)	199.8	172.0	139.5	178.4	200.8	186.2	179.5	218.0	146.6	180.1	182.2	164.5	**45.560**
Food intake (g/b/d)	105.3	92.4	96.6	97.6	102.3	87.9	104.5	108.8	95.5	106.9	104.9	93.8	**14.450**
Total excreta (g/b/d)	109.1	74.1	74.2	91.4	93.8	72.0	101.2	102.2	76.2	89.6	91.1	101.7	**24.390**
Water: feed ratio (g/g)	1.90	1.93	1.44	1.83	1.94	2.19	1.72	1.96	1.51	1.67	1.74	1.77	**0.425**
Water excreted (g/day)	83.2	56.4	51.8	64.3	68.0	53.1	71.9	79.5	58.4	69.0	65.8	77.0	**19.080**
Dry matter excreted (g/day)	25.9	17.7	22.4	27.1	25.8	18.8	29.3	22.7	17.8	20.6	25.3	24.7	**7.027**
Excreta score[2]	5.5	4.7	5.2	4.7	4.7	5.0	6.0	5.2	5.7	5.5	5.0	3.7	**1.060**

1. Error df = 94 (all parameters)
2. See text

Table 7.7. The effects of diets containing different concentrations of differing cereal sources on excreta moisture, water intake and other parameters of laying hens (high fibre diets)

	Diet												
	2	4	6	8	10	12	14	16	18	20	22	24	SEM[1]
Maize	500	375	375	250	250	125	125	.....	.....	.....	.....	.....	
Barley	.....	125	.....	250	.....	.....	375	500	375	250	.....	125	
Wheat	.....	.....	125	.....	250	375	.....	.....	125	250	500	375	
Excreta Moisture (g/kg)	755.1	776.4	754.7	765.0	757.7	801.2	781.1	748.3	786.8	756.5	771.3	803.5	**43.540**
Water intake (g/b/d)	179.6	203.3	218.0	211.7	260.3	251.7	195.9	197.0	177.3	181.1	175.5	195.9	**43.560**
Food intake (g/b/d)	113.6	109.8	109.7	109.4	112.0	108.1	113.2	97.5	106.9	112.5	103.8	107.3	**14.450**
Total excreta (g/b/d)	89.80	113.3	97.9	108.9	106.0	119.4	99.8	99.1	111.7	102.2	90.8	114.6	**24.390**
Water: feed ratio (g/g)	1.67	1.87	2.02	1.91	2.34	2.33	1.72	2.02	1.62	1.60	1.69	1.82	**0.425**
Water excreted (g/day)	68.30	88.00	74.00	83.70	81.20	94.80	58.40	74.50	87.80	77.30	70.10	91.80	**19.080**
Dry matter excreted (g/day)	21.55	25.31	23.88	25.22	24.77	24.61	22.32	24.63	23.95	24.87	20.72	22.88	**7.027**
Excreta score[2]	5.00	3.65	5.25	5.25	5.00	5.50	4.25	4.25	4.225	4.00	4.50	4.25	**1.060**

1. Error df = 94 (all parameters)
2. See text

Table 7.8. The effect of diets containing different concentrations of different cereal sources on some ileal and caecal parameters of laying hens (low fibre diets)

	Diet											
	1	3	5	7	9	11	13	15	17	19	21	23
Maize	500	375	375	250	250	125	125	.....	.....	.....	.....	.....
Barley	.....	125	.....	250	.....	.....	375	500	375	250	.....	125
Wheat	.....	.....	125	.....	250	375	.....	.....	125	250	500	375
Caecal tissue wet weight (g/100g body weight)	0.60	0.76	0.76	0.84	0.83	0.75	0.75	0.97	0.79	0.79	0.85	0.66
Caecal contents (wet weights) (g/bird)	0.80	2.90	2.70	5.20	4.10	2.50	1.90	7.80	1.40	1.30	5.20	4.00
Caecal digesta viscosity (Cp)	2.76	5.11	4.06	1.94	2.86	8.33	2.77	4.42	7.35	3.32	3.77	4.42
Caecal digesta moisture (g/kg)	375.00	482.70	814.80	519.20	634.10	400.00	536.80	579.40	642.80	700.00	711.50	625.00
Caecal digesta water (g/bird)	0..30	1.40	2.20	2.70	5.40	1.75	1.02	4.52	0.89	0.80	3.69	2.50
Caecal digesta dry matter (g/bird)	0.50	1.50	0.50	2.50	4.50	0.75	0.88	3.28	0.50	0.50	1.50	1.50
Ileal tissue wet weight (g/100g body weight)	5.04	5.19	5.21	5.78	4.34	4.81	5.47	5.81	5.39	5.46	4.84	5.09
Ileal contents(wet weights) (g/bird)	3.00	5.40	6.30	3.30	8.20	7.20	4.80	8.60	3.90	9.00	7.90	6.50
Ileal digesta viscosity (Cp)	3.83	6.34	3.89	24.60	6.18	9.32	18.40	10.30	23.90	3.54	9.76	3.84
Ileal digesta moisture (g/kg)	833.30	722.20	821.60	848.40	817.00	791.60	687.50	825.50	871.70	740.70	810.10	923.00
Ileal digesta water (g/bird)	2.49	3.90	5.17	2.79	6.69	5.69	3.30	7.10	3.39	6.49	6.39	5.99
Ileal digesta dry matter (g/bird)	0.50	1.50	1.13	0.50	1.50	1.50	1.50	1.50	0.50	2.50	1.50	0.50

Table 7.9. The effect of diets containing different concentrations of different cereal sources on some ileal and caecal parameters of laying hens (high fibre diets)

	Diet											
	2	4	6	8	10	12	14	16	18	20	22	24
Maize	500	375	375	250	250	125	125	.....	.....	.....	.....	.....
Barley	.....	125	.....	250	.....	.....	375	500	375	250	.....	125
Wheat	.....	.....	125	.....	250	375	.....	.....	125	250	500	375
Caecal tissue wet weight (g/100g body weight)	0.89	0.91	1.12	1.14	0.86	0.83	0.95	0.91	0.85	0.83	0.89	0.87
Caecal contents(wet weight) (g/bird)	3.30	10.10	10.40	11.70	3.40	9.90	3.10	2.50	2.50	5.00	5.00	5.20
Caecal digesta viscosity (Cp)	5.22	4.01	5.01	2.89	2.76	5.70	2.19	2.22	3.04	4.42	3.48	7.60
Caecal digesta moisture (g/kg)	545.40	752.50	759.60	700.50	852.90	545.40	838.70	800.00	700.00	615.40	700.00	519.20
Caecal digesta water (g/bird)	1.80	7.60	7.89	8.19	2.90	5.40	2.60	2.00	1.76	3.50	3.50	2.69
Caecal digesta dry matter (g/bird)	1.50	2.50	2.50	3.50	0.50	4.50	0.50	0.50	0.75	1.50	1.50	2.50
Ileal tissue wet weight (g/ 100g body weight)	5.61	5.77	5.96	5.84	6.19	6.09	6.04	5.66	5.12	5.98	5.09	5.15
Ileal contents(wet weight) (g/bird)	5.50	8.20	1.60	9.10	12.30	3.90	4.90	9.50	5.40	13.50	2.70	3.50
Ileal digesta viscosity (Cp)	3.16	2.61	6.20	24.60	3.86	9.84	14.55	10.30	23.90	17.60	24.90	14.70
Ileal digesta moisture (g/kg)	727.20	817.00	687.50	835.10	796.70	871.70	693.90	736.80	798.10	722.20	444.40	571.40
Ileal digesta water (g/bird)	3.99	6.70	1.10	7.59	9.79	3.39	3.40	6.99	4.31	9.99	1.02	1.99
Ileal digesta dry matter (g/bird)	1.50	1.50	0.50	1.50	2.50	0.50	1.49	2.50	1.09	3.50	1.50	1.50

moisture contents (g/kg) of caecal digesta and increased both caecal and ileal tissue wet weights ($p<0.01$). There were no effects ($p>0.05$) of increased dietary wheat bran concentration on the weights of dry matter or viscosity of caecal digesta or wet weights or weights of water of ileal digesta but a decrease ($p<0.05$) in the moisture content (g/kg) of ileal digesta was observed (Table 7.11.).

Table 7.10. Effect of dietary wheat bran concentration on excreta moisture, water intake and other parameters of laying hens

	Dietary wheat bran concentration (g/kg)			
Parametre	0.00	10.00	SEM[1]	Significance[2]
Neutral detergent fibre (g/kg)	63.50	95.80	**10.180**	***
Water intake (g/bird/d)	174.20	213.00	**43.560**	**
Food intake (g/bird/d)	100.20	108.20	**14.450**	**
Excreta moisture (g/kg)	742.10	772.10	**43.540**	**
Total excreta (g/bird/d)	90.80	103.40	**24.390**	**
Water: feed ratio (g/g)	1.74	1.97	**0.455**	*
Water excreted (g/bird/d)	67.20	80.10	**19.080**	**
Dry matter excreted (g/bird/d)	23.60	23.30	**7.027**	NS
Dropping consistency[3]	4.98	4.72	**1.060**	NS

1. Error df = 94 (all parameters)
2. Significant difference *** ($p<0.001$), ** ($P<0.01$) * ($P<0.05$), NS (Non significant)
3. See text

Table 7.11. Effect of dietary wheat bran concentration on caecal and duodenal characteristics of laying hens

	Dietary wheat bran concentration (g/kg)			
Parameter[1]	0.0	10.0	SEM[2]	Significance[3]
Neutral detergent fibre (g/kg)	62.90	96.30	**9.730**	***
Caecal tissue wet weight (g/100g body weight)	0.79	0.92	**0.105**	**
Caecal contents (wet weight) (g/bird)	3.32	6.01	**2.850**	*
Caecal digesta viscosity (Cp)	4.35	3.95	**1.760**	NS
Caecal digesta moisture (g/kg)	578.00	701.00	**119.000**	*
Caecal digesta water (g/bird)	1.97	4.15	**1.970**	*
Caecal digesta dry matter (g/bird)	1.35	1.85	**1.090**	NS
Ileal tissue wet weight (g/100g body weight)	5.15	5.71	**0.446**	**
Ileal contents (wet weight) (g/bird)	6.18	6.68	**3.075**	NS
Ileal digesta viscosity (Cp)	13.60	14.10	**12.18**	NS
Ileal digesta moisture (g/bird)	806.00	727.20	**97.100**	*
Ileal digesta water (g/bird)	4.96	5.04	**2.501**	NS
Ileal digesta dry matter (g/bird)	1.22	1.63	**0.743**	NS

1. All values adjusted for body weight.
2. Error df = 22 (all parameters)
3. Significant difference *** ($p<0.001$), ** ($p<0.01$), * ($P<0.05$), NS (Non significant)

7.4. DISCUSSION

*7.4.1. **Resistant Starch*** Increased dietary concentrations of resistant starch as a proportion of total starch gave linear increases in water intakes, and the moisture contents of excreta of laying hens (Figure 7.3.). No previous quantitative estimates for the effect of dietary resistant starch concentration on the moisture content of excreta or water intakes could be found. However the observed effects were consistent with the subjective observations of Pattison (1989) and Cooke and Raine (1986) who both suggested that increased resistant starch concentrations were the causative factor of increased litter moistures in broilers fed tapioca and legumes or tapioca respectively. Tapioca however contains a high potassium concentration and therefore the observed effects could have been the result of increased potassium intake. However in this experiment increased dietary resistant starch concentrations gave linear increases in water intakes and excreta moisture when constant concentrations of macro - minerals were maintained. A proportion of the variation in litter moisture observed with legumes and tapioca could therefore be explained by the increased concentrations of dietary resistant starch.

There were small linear increases in daily dry matter excretion with increased concentrations of resistant starch. Cummings *et al* (1996) in man found there to be no effect of resistant starch on water excretion but related the observed laxative effect to increased dry matter content of the faeces due to stimulation of biomass and increased excretion of nitrogen, lipid and carbohydrate. The increase in dry matter observed here could reflect a similar effect of resistant starch in the hen. However unlike in man there were large increases in daily water excretion in the hen. This difference may reflect an effect of resistant starch on reabsorption of water from urine in the fowl.

Increased dietary concentrations of resistant starch will increase the proportion of starch reaching the hindgut (Englyst and Cummings, 1987 b). Leegwater *et al,* (1974) in rats suggested that

potato starch (750 g/kg resistant starch) or other undigested dietary components to have osmotic properties in the hindgut. Increased dietary concentrations of resistant starch could therefore influence water reabsorption from within the faeces and urine. Cummings *et al* (1996) however stated that it was unlikely that resistant starch would hold water in the hindgut since resistant starch is insoluble and has no significant water holding properties. Resistant starch has a non - starch polysaccharide sparing effect when added to the diet, bacteria fermenting resistant starch in preference to non - starch polysaccharide and therefore a small part of the effect on water balance could be attributed to additional non - starch polysaccharide in faeces holding water. This is unlikely to be sufficient to explain the increase in excreta moisture observed here. Although poorly digested resistant starch is readily fermented (Ranhotra *et al,* 1991). Although unclear in the fowl it is known that in mammals carbohydrates are fermented to short chain fatty acids (Topping and Illman, 1986). High concentrations of osmotically active short chain fatty acids may prevent the reabsorption of water from faeces and urine in the hindgut.

Starch is often assumed to be completely hydrolysed and absorbed within the small intestine. It is now known that a proportion of this starch will escape digestion in the small intestine and enter the hindgut where it could be fermented. There is considerable variation in the concentrations of resistant starch in different dietary components (Appendix 7) and in the degree of resistance of various resistant starches (Wyatt and Horn, 1988). Leguminous feedstuffs contain higher concentrations of poorly digested resistant starch compared to cereal based feedstuffs (Englyst *et al,* 1992). Cooking and processing of feeds can increase concentrations of retrograded starch (Longstaff and Mc Nab, 1987). By controlling the quantity and type of dietary ingredients rich in resistant starch as a proportion of total starch used in ration formulation a significant proportion of variation in excreta moisture could be controlled.

7.4.2. Cereal source effects In contrast to previously published work (e.g. Gohl *et al,* 1978; Herstadt, 1987; Marquardt *et al,* 1994) variation in the source and concentration of the cereal fraction of the diets in this experiment had no effect ($p>0.05$) on the excreta moistures or water intakes of the laying hens. Variation in the composition of the cereal source in this experiment failed to give the differences in concentrations of non - starch polysaccharide expected as indicated by the neutral detergent fibre estimate (Table 7.4.). Laying hens have also been reported to be less sensitive to the anti - nutritive effects of increased dietary concentrations of non - starch polysaccharide than broilers (Jeroch, 1987; Johnson, 1987; Campbell and Classen, 1989). The differences between present and other published works were therefore not altogether unexpected.

Although past work has shown that variation in cereal source can influence excreta moisture the present work suggests that the variation in non - starch polysaccharide concentration with variation in cereals used in UK diets with differing dietary formulations may in some cases be too small to have a significant effect on the excreta moisture of layers.

7.4.3. Wheat bran concentration Increased wheat bran concentrations of the diet increased excreta moistures and water intakes of the laying hens. The wheat bran effects could not be attributed to any one nutrient although correlations between excreta moisture and neutral detergent fibre suggest the insoluble fibre of wheat bran may be a factor related to the increased excreta moisture of laying hens. Insoluble non - starch polysaccharide is almost completely undigested in the fowl (Carre *et al,* 1990) and fermentation is poor (Pettersson and Aman, 1989). There may be osmotic effects of such poorly absorbed compounds in the hindgut as observed in rats (Leegwater *et al,* 1974) and pigs (Cooper and Tyler, 1959). Present data however contrasts with that of Jorgensen *et al* (1996) in broilers who found no effect of increased wheat bran concentration on excreta moisture but found increased wet weights of excreta. Wheat bran is a milling fraction of

wheat and subject to variation in composition. As well as containing high concentrations of insoluble non - starch polysaccharide it may contain varying concentrations of soluble non starch polysaccharide depending on the level of endosperm contamination. Soluble non - starch polysaccharides are known to increase excreta moisture (e.g. Choct and Annison, 1992 a and b). Differences in the concentrations of soluble non - starch polyssacharide concentration may explain the difference between these and previously published data.

Increased dietary concentrations of wheat bran increased tissue wet weights of both the caeca and ileum of the hens consistent with the hypertrophy in gut tissues seen in previous studies of birds when fed increased concentrations of dietary fibre (Savory and Gentle, 1976; Jorgensen *et al,* 1996). Increased caecal size could reflect the increased bulk of caecal digesta although Clemens *et al* (1975) suggested insoluble material would have difficulty in entering the caeca of birds. In line with Jorgensen *et al* (1996) increases in mean tissue weights were proportionally greater for caeca (146 g/kg) than ileum (98 g/kg) with increased wheat bran concentrations.

There were no effects of dietary wheat bran concentration on the viscosity of either caecal or ileal digesta. Increased ileal digesta viscosities have been related to increased concentrations of soluble non - starch polysaccharides (Bedford, 1995). The soluble fraction represents only a small fraction (<10 %) of total non - starch polysaccharide concentration of wheat bran and measured values for individual birds were low and highly variable as previously observed for older birds (Petersen *et al,* 1993). A lack of an effect was therefore not unexpected.

7.5. CONCLUSION

In conclusion the effect of increasing the concentration of dietary resistant starch, as a proportion of total starch, and independently of variation in electrolyte concentrations of laying hens, was to give a linear increase in the moisture contents of excreta. There may therefore be economic benefits to laying hen flocks of reducing the dietary concentrations of components containing high concentrations of resistant starch as a proportion of total starch. Diets in experiment 11 failed to give the variation in non - starch polysaccharide concentration expected. There were therefore no effects of cereal source on excreta moisture of laying hens in contrast to previous published reports. Increased dietary concentrations of wheat bran increased excreta moistures of the hens. Although the causative factor in wheat bran was unclear, there were significant correlations between neutral detergent fibre concentration of the diets and excreta moisture which suggests that the insoluble fibre concentration may be one component of wheat bran that affects excreta moisture.

CHAPTER 8.

THE EFFECT OF DIETARY FAT CONCENTRATION AND COMPOSITION ON EXCRETA MOISTURE AND WATER INTAKES OF LAYING HENS

8.1. INTRODUCTION

Birds can assimilate relatively large amounts of a wide range of dietary fats, however there are large divergences in the efficiencies with which various fats, are digested by the fowl (Freeman, 1976). A number of factors influence the assimilation of dietary fat, of which the dietary fat composition which has physico-chemical effects on both the digestive and absorptive process and the fat concentration are of primary importance (Freeman, 1976). The greater the degree of unsaturation of a fat the greater its digestibility (Freeman, 1976) and the greater the concentration of fat in the diet the lower the digestibility (March and Biely, 1957; Fedde *et al,* 1960).

Rancidity of fats can occur and is of two types; oxidative and hydrolytic. Hydrolytic rancidity occurs from the action of microorganisms causing a simple hydrolysis of the oil and fails to significantly affect nutritional value (Scott *et al,* 1982). In contrast, oxidative rancidity reduces the digestibility and energy value of unsaturated fatty acids (Pattison, 1989) through a free radical mechanism which produces poorly absorbable linear and cyclic hydro peroxides (Wiseman, 1986).

Any part of dietary fat that is not digested must be excreted in the form of faecal fat and could therefore affect the composition of excreta. Bray (1985) has shown that excess fat in broiler diets results in litter which is both lower in moisture and greasy. However the lowered moisture content of the litter may however not reflect decreased moisture contents of excreta but increased evaporation, following capping. This is supported by Patrick and Ferrise (1962) who have shown that birds fed increased fat concentrations failed to increase their water intakes, although Jordan (1990) has stated that poor quality fat, or fat poorly digested by poultry will lead to diarrhoea.

As there is little quantitative information that describes the variation in excreta moisture with different concentrations of dietary fat, or to variation in the quality of fat in laying hens, an experiment was carried out to quantitatively measure the response in water intake and excreta moisture of laying hens to dietary concentrations of fat. A second objective of the experiment was to examine for fat concentration x fat source x fat oxidation level interactions when either predominantly saturated (tallow) or un-saturated (soyabean oil) fats were fed, either with, or without, prior exposure to oxidising conditions.

8.2. MATERIALS AND METHODS

8.2.1. Experiment 12 Sixty four, 32-week-old ISA Brown laying hens were caged in individual wire floored cages (50 x 45 x 45 cm) arranged in four tiers, within an environmentally controlled room. Each cage had an individual feed hopper, water trough and excreta collection tray. The birds were maintained under a 14L : 10D lighting regime at 24 ±1 °C and 80 ±5 % relative humidity.

Sixteen practical laying hen diets (12.0 MJ/kg of ME and 166.0 g/kg of crude protein) that were identical in nutrient composition (Table 8.1.) were compared, in which one of four concentrations of supplementary dietary fat (60, 90, 120 and 160 g/kg) provided as either soyabean oil (fatty acids pre-dominantly un saturated) (Appendix 9) or tallow (fatty acids pre-dominantly saturated) (Appendix 9), each, either with or without prior exposure to oxidising conditions (preperation as described below) were added. Fat concentrations were increased by replacement for sucrose and maize starch, to maintain equivalent metabolisable energy contents of diets, and washed sand. Macro - mineral concentrations were maintained constant by replacement of washed sand with inorganic mineral salts. Diets were mixed not more than 6 h before the start of the experiment to limit decomposition of hydroperoxides during storage. Each bird had *ad libitum* access to one of the sixteen diets and water throughout the eight day feeding period.

Water intakes and feed intakes were measured, and all excreta were collected on the final two days of the feeding period. Excreta were collected in open trays, and excreta moisture levels determined by drying total excreta at 60 °C in a forced air oven. Data was corrected for loss of moisture to the environment using the correction factor obtained in experiment 1. Water and feed intakes were determined by weighing the appropriatte trough at the beggining and the end of the collection period. Water intakes were adjusted for loss of water vapour from the drinkers. Corrections were made by determining the amount of water vapour lost from identical drinkers situated on each tier

in a possition innacesable to the laying hens.Only on egg laying days was data used in calculation of treatment means, to reduce variation as a result of a response in water balance to egg formation (Wood- Gush and Horne, 1970).

On day eight excreta were scored according to their consistency. The scoring system placed excreta into six divisions according to the consistency of the excreta from (1) pasty to (6) particulate. Mean values were determined for each diet.

The experiment was designed as a randomised block analysis of variance, with cage tier level as a blocking factor. Fat concentration and fat concentration x fat source x oxidation level interaction sums of squares were partitioned into a set of orthogonal linear and quadratic polynomial regression components using the GENSTAT statistical package (Lawes Agricultural trust, 1984). Multiple regression analyses, with water intake and feed intake as independant variables, and excreta moisture as a dependent variable, were also carried out.

8.2.2. Preparation and characterisation of the fats used Samples of the soya oil and tallow were obtained which had iodine values of 139 and 6 and peroxide values of 1.18±0.003 and 1.20± 0.009 m Eq/kg respectively. Proportions of each were exposed to oxidising conditions by continuously bubbling through oxygen whilst the oil was left at 90±2 °C for 48 h, according to the method of L' Estrange *et al* (1966). The peroxide values increased to 140.54±0.409 and 18.64±0.046 m Eq/kg for soyabean oil and tallow respectively. Fats were stored, refrigerated at -5±1 ° C to reduce loss of hydroperoxide, and the tallow was ground through a 0.5 mm mesh screen prior to incorporation into diets.

Each of the fats were characterised by determination of their iodine absorption number according to the Wijs method, and peroxide value using a titrimetric method, both according to A.O.A.C.

Table 8.1. Composition (g/kg) and analysis of basal diet for experiment 12

Component	g/kg diet
Wheat	200.0
Maize gluten meal	64.0
De-hulled soyabean meal	150.0
Sunflower meal	110.0
Soya bean oil	50.0
Limestone	86.0
Sucrose[1]	150.0
Sand[1]	20.0
Cornflower[1]	150.0
Vitamin mineral premix[2]	20.0
Analysis (calculated)	
Crude fat	59.5
Crude protein	166.0
Lysine	7.1
Methionine	4.2
Calcium	38.5
Phosphorus	4.0
Sodium	1.6
Potassium	5.9
Metabolisable energy (MJ of ME/kg)	12.0

1. Sand, sucrose and cornflower used as fillers in formulation of experimental diets to maintain constant ME concentration

2. Comprised ash (817 g/kg), calcium (200 g/kg), phosphorus (113.0 g/kg), methionine (50 g/kg), sodium (75 g/kg), copper (cupric sulphate 250 mg/kg), vitamin A (300000 i.u./kg), vitamin E alpha tocopherol acetate (300 i.u./kg), vitamin D3 (150000 i.u./kg)

(1990). At the end of the experimental period, diets were subject to soxlet extraction of fat, samples of which were analysed for peroxide value, to determine the loss of hydro-peroxide during the experimental period.

8.2.3. Feed and excreta analyses Feeds for each experiment were ground to pass a 0.5 mm mesh screen and were subsequently analysed by standard proximate analysis (A.O.A.C., 1990). Sodium, calcium, phosphorus and potassium concentrations were determined with an atomic absorption spectrophotometer (Smith-Hieftje 1000, Thermo Jarrell Ash Corporation) following wet digestion (A.O.A.C., 1990). Inorganic phosphorus was determined using a colorimetric technique (MAFF, 1986) in which concentration of phosphorus in a trichloroacetic acid extract is determined spectrophotometrically as the yellow phospho- vanado- molybdate complex at 400 nM. Diets which failed to conform to the calculated ananlysis +/- 10% (Table 8.1.) were re-mixed and re-analysed

Total excreta following drying were ground to pass a 0.5 mm mesh screen. Samples were subsequently analysed for fat concentration using soxhlet extraction with petroleum ether (A.O.A.C. 1990).

8.3. RESULTS

8.3.1. Crude fat concentration effects (see Table 8.2.) There were no effects ($p>0.05$) of dietary crude fat concentration on feed intake, so increased dietary crude fat concentrations produced linear increases in fat intake ($p<0.001$). Increased dietary crude fat concentrations gave linear decreases ($p<0.01$) in dry matter digestibility, but linear increases ($p<0.001$) in dietary fat digestibility and therefore there were no differences ($p>0.05$) in the quantity of fat excreted. There were fat type x fat concentration ($p<0.01$) and oxidation level x fat concentration interactions ($p<0.01$) for fat digestibility. However there were no effects ($p>0.05$) of dietary fat concentration and no interactions ($p>0.05$) for water intakes, water to feed intake ratios, weights of water excreted or excreta score, but there were linear decreases in the moisture contents of the excreta ($p<0.001$) and a linear increase in total excreta (wet weight).

8.3.2. Fat source effects (see Table 8.2.) The soya oil had a greater ($p<0.001$) iodine absorption value than tallow and therefore contained a higher proportion of unsaturated fatty acids, as expected. There were no effects ($p>0.05$) of fat type on feed intake, water intakes, water to feed intake ratios, weights of water excreted, total outputs (fresh weights) or moisture contents of the excreta. However saturated fat had a lower digestibility ($p<0.01$) than unsaturated fat, which increased excretion of dietary fat ($p<0.001$) and lowered the excreta consistency score ($p<0.01$).

8.3.3. Effect of peroxide value (see Table 8.2.) Exposure of fat to oxidising conditions increased ($p<0.001$) the peroxide value of both soyabean oil and tallow. There was a saturation level x oxidation level interaction ($p<0.001$) for peroxide value. Storage of diets for eight days reduced the peroxide value by 8 % (saturated fat) and 4% (unsaturated fat). There were no effects ($p>0.05$) of fats with higher peroxide values on feed intakes, water intakes, water to feed intake ratios,

weights of water excreted, total outputs (fresh weight) of excreta or moisture contents (g/kg) of the excreta. However oxidation of dietary fat reduced ($p<0.05$) digestibility of dietary fat, increased excretion of fat ($p<0.05$) and therefore lowered ($p<0.05$) excreta consistency score. There were saturation x oxidation level interactions ($p<0.05$) for fat digestibility and excreta consistency score.

Table 8.2. Effect of dietary concentration, level of saturation and level of oxidation of fat on the excreta moisture, water intake and other parameters of laying hens

		Dietary fat concentration (g/kg)													
		Saturated fat[1]				Unsaturated fat[2]					Significance[4]				
		60	90	120	160	60	90	120	160	SEM[3]	Concentration	Saturation	Oxidation	Oxidation x saturation	Oxidation x concentration	Saturation x concentration
Food intake (g/b/day)	Oxidised	132.10	139.20	143.80	143.70	125.90	145.40	143.70	136.40							
	Unoxidised	143.30	139.60	145.20	152.80	133.10	146.60	142.80	149.50	17.640	NS	NS	NS	NS	NS	NS
Fat intake (g/b/day)	Oxidised	7.92	12.53	17.26	22.99	7.55	13.08	17.24	21.82							
	Unoxidised	8.60	12.57	17.43	24.45	7.98	13.19	17.14	23.92	2.109	***	NS	NS	NS	NS	NS
Fat excreted (g/b/day)	Oxidised	1.78	2.26	2.22	1.74	0.69	1.39	1.15	1.37							
	Unoxidised	1.47	2.00	2.14	1.77	0.48	0.74	0.86	1.29	0.519	NS	***	*	*	NS	NS
Apparent fat digestability (%)	Oxidised	81.67	83.66	87.54	92.26	90.61	90.07	93.38	93.75							
	Unoxidised	78.98	82.07	87.29	92.88	93.96	94.23	94.70	94.34	4.160	***	***	*	*	NS	**
Dry matter digestibility (%)	Oxidised	72.00	67.00	63.00	67.00	76.00	70.00	69.00	64.00							
	Unoxidised	73.00	70.00	70.00	70.00	76.00	74.00	74.00	63.00	4.800	***	NS	NS	NS	NS	*
Water intake (g/b/day)	Oxidised	200.40	214.50	235.90	229.10	219.90	214.50	243.30	206.20							
	Unoxidised	210.90	228.20	223.50	226.20	210.40	210.80	221.80	232.90	26.850	NS	NS	NS	NS	NS	NS
Water: feed intake (g/g)	Oxidised	1.51	1.55	1.64	1.61	1.72	1.46	1.72	1.51							
	Unoxidised	1.48	1.64	1.55	1.48	1.58	1.45	1.59	1.59	0.194	NS	NS	NS	NS	NS	NS
Excreta moisture (g/kg)	Oxidised	645.80	616.10	622.80	633.30	683.20	647.20	647.00	616.30							
	Unoxidised	677.60	637.10	617.70	618.00	675.40	641.40	647.00	594.70	32.520	***	NS	NS	NS	NS	NS
Total excreta (g/b/day)	Oxidised	104.60	121.60	140.00	129.90	97.00	129.80	125.00	127.60							
	Unoxidised	121.30	113.90	112.00	118.80	102.80	104.60	102.00	131.50	22.930	NS	NS	NS	NS	NS	NS
Water excreted (g/b/day)	Oxidised	67.40	75.00	87.20	82.30	66.70	85.50	81.00	77.90							
	Unoxidised	82.20	72.50	68.80	73.40	70.40	67.20	66.10	78.20	16.040	NS	NS	NS	NS	NS	NS
Dry matter excreted (g/b/day)	Oxidised	37.20	46.60	52.80	47.60	30.30	44.30	44.10	49.70							
	Unoxidised	39.10	41.30	43.20	45.40	32.40	37.40	36.10	53.30	8.170	***	NS	NS	NS	NS	NS
Excreta score	Oxidised	3.00	3.25	3.25	3.25	4.50	3.25	3.25	3.00							
	Unoxidised	4.75	3.25	3.25	3.25	4.25	4.25	4.25	4.50	0.586	NS	**	*	*	NS	NS

1. Beef tallow
2. Soya oil
3. Error df = 45 (all parameters)
4. Significance *** (p<0.001), ** (p<0.01), * (p<0.05), NS (p>0.05)

8.4. DISCUSSION

8.4.1. Fat concentration Increased dietary fat concentration produced a linear decrease in the moisture contents of excreta of the hens but had no effect on water intakes. No previous estimates for the effect of dietary fat concentration on excreta moisture were found although previous observations of Patrick and Ferrise (1962) have shown increased dietary fat concentrations to have no effect on water intakes of broilers consistent with this experiment. A more detailed examination of the present data showed that the decrease in excreta moisture observed was explained by a linear decreases in dry matter digestibility of diets with increased dietary fat concentration. Previously published works have suggested dry matter digestibility to be either increased, due to decreased rates of feed passage (Krogdahl, 1985 a), or unaffected by dietary fat concentration (Renner and Hill, 1960) when constant metabolisable energy contents are maintained. Numerous workers have observed reduced digestibilities of minerals with increased dietary fat concentrations due to soap formation. However although calcium concentrations of laying diets are high Atteh and Leeson (1985) have shown that soap formation occurs post absorbitivly in laying hens. Variation in fat concentrations of diets would therefore be expected to give consistent dry matter digestibilities and no effect on excreta moistures. Data obtained here therefore do not support an effect of dietary fat concentration on excreta moisture of laying hens in diets found in commercial practice.

In agreement with previous work (Atteh and Leeson, 1985) there were linear increases in fat digestibility with increased dietary fat concentration. There were therefore no effects of dietary fat concentration on fat excretion and therefore no effect on the consistency of the excreta.

8.4.2. Fat quality There were no effects of fat quality in terms of the degree of fatty acid

saturation or oxidation on the excreta moisture or water intakes of laying hens. No previous estimates for the effect of either on excreta moisture were found although Jordan (1990) has stated that reduced fat digestibility would lead to diarrhoea in poultry. Increased levels of saturation of dietary fat and oxidation both reduced the fat digestibility consistent with previous observations (Freeman, 1976) and saturation x oxidation interactions reflected the increased sensitivity of unsaturated fat to oxidation. However the failure of the variation in fat digestibility achieved in this experiment (despite levels of oxidation higher than allowed in commercial practice) to have an effect on excreta moisture may suggest that increased fat excretion has no effect on moisture content of excreta in laying birds.

The excreta score was greater for saturated fat than un - saturated fat and increased when fat was exposed to oxidation although there were saturation x oxidation level interactions. These observations are consistent with the trends in fat digestibility. Increased concentrations of fat in the excreta may therefore render excreta pasty. This may reduce the economic efficiency of a laying flock through an increase in the proportion of dirty eggs.

8.5. CONCLUSION

In conclusion there is no evidence to suggest that increased concentrations of dietary fat or variation in fat quality in terms of level of saturation or oxidation of dietary fat at concentrations found in commercial diets will increase the moisture contents of excreta of laying hens. However aspects of fat quality which reduce fat digestibility may have a detrimental effect on the consistency of excreta.

CHAPTER 9.

THE INFLUENCE OF RAISING EXCRETA MOISTURE BY DIET ON THE NUMBER OF EXCRETA CONTAMINATED EGGS FROM CAGED LAYING HENS

9.1. INTRODUCTION

The proportion of dirty (excreta contaminated) eggs produced by laying flocks are a continuing problem and a major cause of economic loss in the U.K. industry, as under European community (EC) marketing regulations, class A eggs should have a clean shell and have not been cleaned (Anon, 1993 b), washed and soiled eggs being downgraded.

There are potential dangers to human health of using eggs contaminated with excreta (Anon, 1988). Contamination of the egg shell by excreta increases risk of contamination of internal contents by bacterial organisms (Humphrey *et al*, 1989). Various workers have shown a relationship between dirty eggs and increased microbial contamination (Johns & Berard, 1946; Forsyth *et al*, 1953). There are also risks of cross contamination to clean eggs either directly, or through handling.

Previous chapters have shown that dietary composition is a major factor influencing the level of excreta moisture produced by laying hens. Previous subjective observations suggest variation in excreta moisture could affect the number of dirty eggs (Rosenberg, 1955; Herstadt, 1987; Walsh, 1993; Kjaer 1994). Variation in dietary formulations, mixing errors, and variation in the chemical composition of feed ingredients used in diets can cause variation in the excreta moisture of laying hens, and could therefore have economic consequences in terms of increased egg down grading through increased numbers of dirty eggs. The effects of variation in excreta moisture due to diet on the number of dirty eggs needs to be described.

There is little information that quantitatively describes the increase in the number of dirty eggs when the moisture content of the excreta is raised by diet. An experiment involving 1440 caged laying birds was therefore carried out to quantify the change in output of dirty eggs, and the

amount of microbial contamination of egg shells, with increased excreta moisture. Changes in excreta moisture were achieved by feeding different concentrations of dietary sodium.

9.2. MATERIALS AND METHODS

9.2.1. Bird management One thousand four hundred and forty, 38-week-old ISA Brown laying hens were obtained from a commercial flock, previously fed a proprietary layer diet (Appendix 10). They were housed for 3 months in groups of 5 birds, in cages (45 x 45 x 50 cm) arranged in 24 experimental units, of 60 birds each, in 3 tiers. A 14L : 10 D lighting regime was used throughout. Temperature and relative humidity were monitored twice daily. Relative humidity for the house was 67.3±1.56% and daily minimum and maximum temperatures were 16.8±0.47 °C and 20.7±0.30 °C respectively.

9.2.2. Diets Practical laying hen rations (11.80 MJ/kg of ME and 169 g/kg crude protein) were used. They were identical in nutrient composition except for 4 different concentrations of sodium (1.6, 5.0, 10.0 or 15.0 g/kg) as sodium chloride (Table 9.1.). Sodium chloride replaced washed sand in the diet. Birds had *ad libitum* access to water and one of the 4 diets. Each group of five birds had access to 2 nipple drinkers.

9.2.3. Egg and feed measurements Feed intakes were determined weekly for each experimental unit. The numbers of total eggs, cracked and dirty eggs, and egg weights were recorded daily. Dirty eggs were scored (weekly) according to their degree of contamination. The scoring system placed eggs into 2 divisions; these were either contamination with faecal matter or contamination with urates. Each division had 3 subdivisions relating to percentage of the shell surface area contaminated: 0-20%, 20-40% and >40% contamination with either faecal matter or urates.

Total (viable) bacterial counts were carried out on shells of 768 clean intact eggs, 8 from each experimental plot, on 1 day each week, for 4 weeks. All eggs were removed from the collection

Table 9.1. Composition (g/kg diet) and analysis of basal diet for experiment 13

Component	(g/kg)
Wheat	447.00
Barley	189.00
Maize gluten	79.80
Dehulled soya bean meal	36.10
Fish meal	21.50
Meat and bone meal	34.80
Lysine hydrochloride	4.30
Methionine	1.50
Soya oil	43.50
Limestone	75.00
Dicalcium phosphate	5.00
Sodium chloride/sand premix[1]	50.00
Vitamin mineral premix[2]	12.50
Analysis (calculated)	
Sodium	1.60
Potassium	4.00
Calcium	40.00
Phosphorus	4.00
Crude protein	169.00
ME (MJ of ME/kg)	11.80

1. Sand filler was replaced by sodium chloride in preparation of treatment diets. The following ratios of sand: salt were used (5.0 g/kg Na = 4.12 : 0.88; 10.0 g/kg Na = 3.44 : 1.56; 15.0 g/kg Na = 2.82 : 2.17)
2. Comprised ash (890.0 g/kg), calcium (250.0 g/kg), methionine (80.0 g/kg), sodium (88.0 g/kg), copper (cupric sulphate 400 mg/kg), vitamin A (480000 i.u./kg), vitamin E (alpha tocopherol acetate 480 i.u./kg), vitamin D3 (240000 i.u./kg)

cradle of the cage before most of the days eggs were laid. The first 8 clean eggs subsequently laid from each experimental unit were then collected, and placed in individual sterile plastic containers. Each egg was weighed, and the surface area calculated according to the equation of Dunn (1923). The eggs were subsequently refrigerated at +4±1 °C for 24 h. Twenty millilitres of sterile 10% Ringers solution were transferred to each egg container and each was shaken for 30 minutes at 200 strokes per minute, to remove all organisms. Preliminary tests showed that there were no further organisms removed from the shell by prolonging the shaking. Microbial suspensions were serially diluted and pour plates of an appropriate dilution were carried out, using sterile nutrient agar pH 7.4 followed by incubation at 32±1 °C for 24 h.

9.2.4. Feed and excreta analyses Samples of feeds were ground to pass a 0.5 mm mesh screen and were subsequently analysed by standard proximate analysis techniques (A.O.A.C., 1990). Sodium, calcium and potassium concentrations were determined with an atomic absorption spectrophotometer (Smith-Hieftje 1000, Thermo Jarrell Ash Corporation) following wet digestion (A.O.A.C, 1990). Inorganic phosphorus was determined using a colorimetric technique (MAFF, 1986) in which concentration of phosphorus in a trichloroacetic acid extract was determined spectrophotometrically as the yellow phospho- vanado- molybdate complex at 400 nM.

Excreta collections for moisture determinations were carried out weekly for 24 h. Collection involved placing 3 pre-weighed aluminium trays at randomly selected positions on the manure belt, for each experimental unit. The moisture contents of the excreta were determined by drying at 60 °C in a forced air oven.

9.2.5. Statistical analyses The experiment was designed as a randomised block with a blocking factor of 3 cage tier levels. Weekly data were collected for each experimental unit. To test for a

relationship between excreta moisture and the number of dirty eggs, and microbial contamination of first quality egg shells, linear regression coefficients were obtained by fitting data to a simple linear model. A second regression analysis used the diet factors as dummy variables in a model that assumed parallel regression coefficients between different diet groups. Viable count data were transformed logarithmically. The proportions of eggs scored for severity of excreta contamination were analysed by Chi-square analysis of proportions technique.

9.3. RESULTS

There were no effects of dietary sodium concentrations ($p>0.05$) on total egg numbers (Table 9.2.), but a linear decrease in mean egg weight occurred ($p<0.05$). Increased dietary sodium concentrations had no effect ($p>0.05$) on feed intakes (Table 9.2.) and therefore gave linear increases ($p<0.001$) in daily sodium intakes (Table 9.3.). There were linear increases ($p<0.001$) in moisture contents of the excreta (g/kg) with diet (Figure 9.1.).

Each 10 g/kg increase in excreta moisture gave a 0.037 (±0.0042) increase ($p<0.001$) in the proportion of dirty eggs between treatments (Figure 9.2.) and 0.050 (±0.021) increase within a treatment ($p<0.05$). There were no differences ($p>0.05$) between these two estimates of the regression coefficients. Each 10 g increase in excreta moisture also gave a 0.0081(±0.00094) increase ($p<0.001$) in the microbial contamination (log no./cm^2) of first quality egg shells between treatments (Figure 9.3.) and a 0.0131 (±0.0051) increase ($p<0.05$) within a treatment. There were again no differences ($p>0.05$) between the two regression coefficients. No attempt was made to identify the microbial flora present although it was observed to be heterogenous in nature.

The ratio of eggs contaminated with faecal matter, to eggs contaminated with urates, did not change ($p>0.05$) with moisture content of the excreta (Table 9.3.). The severity of contamination with faecal matter increased ($p<0.01$) with increased excreta moisture (Table 9.3.). A trend ($p>0.05$) was observed towards an increase in the degree of contamination of the urate contaminated eggs with excreta moisture, but this failed to reach statistical significance (Table 9.3.).

Table 9.2. Effects of dietary treatments in experiment 13 on flock production parameters

	Dietary sodium (g/kg)					
	1.60	5.00	10.00	15.00	SEM[1]	Significance[2]
Egg numbers (eggs/100 birds/d)	88.3	86.8	84.9	84.9	2.94	NS
Mean egg weight (g)	64.8	64.7	64.0	63.2	0.85	*
Feed intake (g/bird/d)	109.1	110.7	110.1	107.6	8.45	NS

1.Error degrees of freedom=18

2.Significance of the regression coefficient (b) where $y=a\pm bx$ *($p<0.05$), NS ($p>0.05$)

Table 9.3. Effect of dietary sodium concentrations on excreta moisture, production of dirty (excreta contaminated) eggs, shell excreta contamination score and the microbial contamination of egg shells with no obvious excreta contamination

		Dietary sodium (g/kg)					
		1.60	5.00	10.00	15.00	SEM[1]	Significance[2]
Sodium intake (g/kg)		1.7	5.5	11.0	16.1	0.93	***
Excreta moisture (g/kg)		732.7	768.0	836.1	865.5	9.70	***
Dirty eggs (%)		6.5	9.0	11.2	10.9	1.99	***
Total bacterial count[3] (no/cm^2)		40.2	49.4	77.2	107.0	0.34[4]	***
Ratio of faecal contaminated: urate contaminated eggs		0.76	0.90	0.84	0.86	0.274	NS
Eggs contaminated	-0-20%coverage	0.57	0.80	0.76	0.88		**[5,6]
with faeces (eggs/100birds/day)	-20-40%	0.05	0.26	0.34	0.22		
	- >40%	0.07	0.12	0.22	0.16		
Eggs contaminated	-0-20% coverage	0.96	1.29	1.45	1.44		NS[5,6]
with urates (Eggs/100birds/day)	-20-40%	0.02	0.05	0.12	0.07		
	->40%	0	0.02	0.02	0.03		

1. Error degrees of freedom=18
2. Significance level of the regression coefficient (b) where y=a+bx *** ($p<0.001$), NS ($p>0.05$)
3. Total bacterial counts expressed per unit area of shell surface
4. SEM for logorithmically transformed data
5. **($p<0.01$), NS ($p>0.05$)
6. Error degrees of freedom for faecally contaminated and urate contaminated eggs are 12 and 6 respectivly

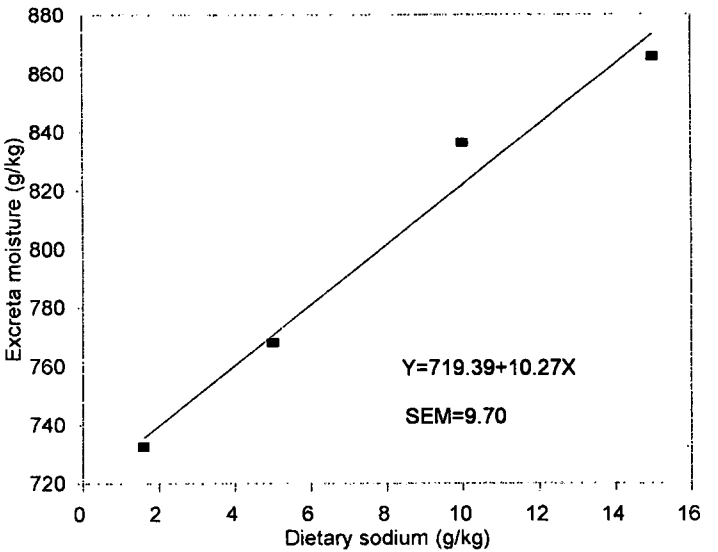


Figure 9.1. The effect of dietary sodium concentration on the excreta moisture of a flock of laying hens

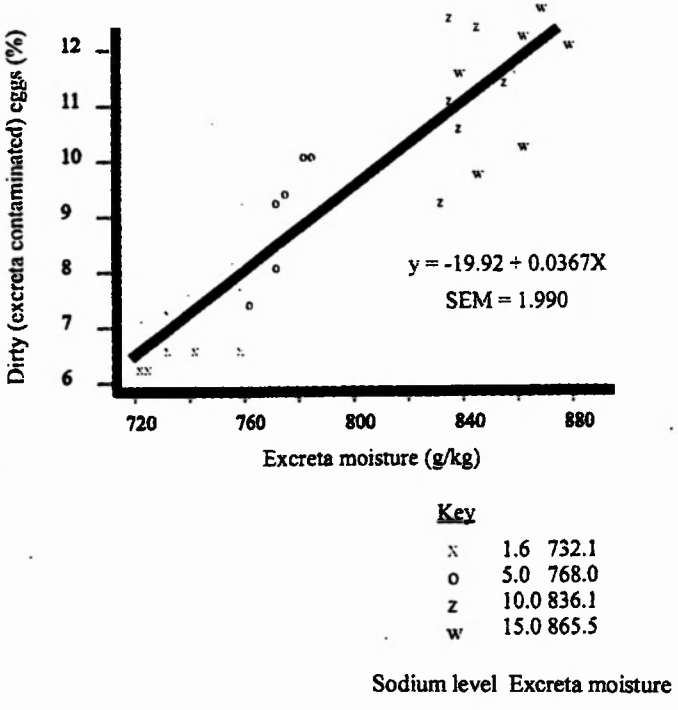
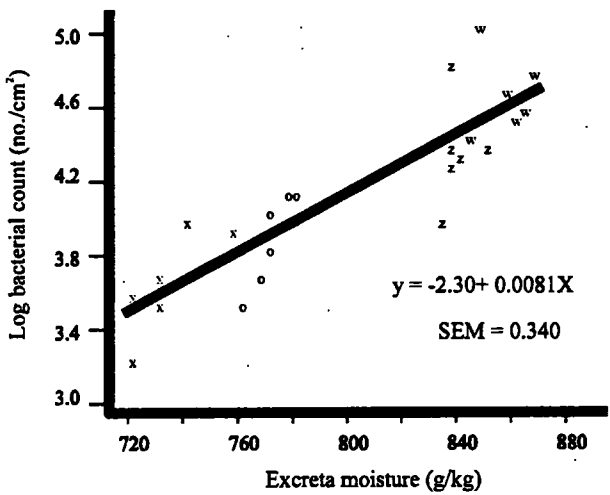


Figure 9.2. The effect of excreta moisture on the proportion of dirty eggs produced by laying hens



Key

x	1.6	732.1
o	5.0	568.0
z	10.0	836..1
w	15.0	865.5

Sodium level Excreta moisture

Figure 9.3. The effect of excreta moisture on the microbial contamination of first quality egg shells

9.4. DISCUSSION

Increased dietary sodium concentration raised excreta moisture of the flock of laying hens to a slightly greater extent than observed previously (Figure 9.1.) for individually caged layers (Smith *et al.* 1997 a). The estimate may have been greater as food consumption did not decrease with the prolonged exposure to sodium, whereas a large decrease in food consumption occurs when excess sodium is fed for a short duration (Damron and Kelly, 1987).

9.4.1. Dirty Eggs Increased moisture content of the excreta of the hens produced a linear increase in the proportion of dirty eggs, which agrees with the subjective observations of Rosenberg (1955). The similar regression coefficients, obtained within and between the four dietary treatments, indicated that excreta moisture was a factor that directly affected the proportion of dirty eggs produced. A high proportion of dirty eggs reduces the economic efficiency of a flock of laying hens. Dirty eggs are, on average, marketed for approximately one third of the price of first quality eggs. A 100 g/kg increase in the moisture content of excreta, for example, would therefore result in a 2.5 % reduction in egg income from a flock.

The contamination of the eggs could have occurred prior to being laid, because the cloaca receives the excreta from both the digestive and urinogenital system. However simultaneous evacuation of faeces during egg laying would be prevented by closure of the mucosal fold between the copradeum and urodeum. Contamination of the eggs would therefore be more likely to occur on the cage floor. Evacuation of excreta has been reported to be more frequent as the moisture content increases (Jordan, 1990), which could increase the probability of soiling of the egg as it passes to the collection cradle. Excreta with a high moisture content will also increasingly adhere to the cage floor, bird feet and feathers, and egg shell surfaces.

Only 76 % of the variation in dirty egg numbers was accounted for by variation in excreta moisture, and within a treatment there was a range in the incidence of dirty eggs of up to four percentage points. Variation in excreta moisture due to diet is therefore not the only factor influencing egg dirtiness. Mineral composition of drinking water, behaviour, environmental factors and disease are also known to influence excreta moisture.

9.4.2. Microbial contamination of first quality egg shells Increased moisture content of the excreta produced a linear increase in the microbial contamination of apparently uncontaminated egg shells. The similar regression coefficients observed within and between the four dietary treatments indicated that excreta moisture was the factor that directly affected the degree of contamination of first quality egg shells.

The bacterial numbers measured in this experiment were low. However the eggs collected in this experiment were only exposed to the environment for a maximum of 2 hours. In commercial situations there would be longer periods in the house, so raising contamination further, and increasing the chance of contamination of internal egg contents.

Micobial contamination is unlikely to have occurred prior to the egg being laid, as Stuart and McNally (1943) found minimal contamination of the egg shell at oviposition. Harry (1963) linked the bacterial content of the shell to the laying environment. Excreta with a high moisture content could increase both the relative humidity within the house and the contamination of cage surfaces, feet and feathers, with excreta. Both conditions would favour microbial proliferation in the cage environment (Jordan, 1990; Esmay and Dixon, 1986) and therefore increase the probability of contamination of eggs during passage to the collection cradle, whilst leaving a superficially clean egg. Increased external populations of a bacteria capable of invading the egg could increase the probability of contamination of the internal contents of first quality eggs (Florian and Trussell,

1957). Faecal material favours proliferation of gram negative bacteria which are able to penetrate shell and membrane more succesfully than those of a gram positive nature (Board, 1968).

9.5. CONCLUSIONS

In conclusion, increases in moisture content of excreta caused by diet will give linear increases in both the number of dirty eggs produced, and the microbial contamination of the shells of eggs. Increased excreta moisture could therefore result in economic loss to the producer through shell egg downgrading and also result in a poorer microbial status of all the eggs produced by a flock.

CHAPTER 10.

GENERAL DISCUSSION

The original objective of this project was to determine quantitatively the effect of dietary nutrient composition on the excreta moisture and water intake of laying hens and to investigate the effect of variation in excreta moisture on the proportion of dirty eggs produced by caged laying hens. A series of experiments with individually caged laying hens examined quantitatively the effects of the major dietary nutrients in diets that varied in only one component. A further experiment involving 1440 caged laying birds examined quantitatively the effect of variation in excreta moisture produced by diet on the proportion of dirty eggs produced by laying birds and also investigated the effect on microbial contamination of eggs. The quantitative estimates obtained for each of the experiments are summarised in Tables 10.1., 10.2. and 10.3.

This chapter attempts to summarise the main findings from this series of experiments, draw some conclusions and subsequently combines the experimental data in a simple model to determine the proportion of variation in dirty eggs from commercial laying hen flocks which can be predicted by variation in dietary nutrient composition, assuming that the relationships determined in this work describe fully the variation in excreta moisture and dirty eggs with diet.

Variation in dietary nutrients in excess of assumed requirements gave large levels of variation (550 to 900 g/kg) in excreta moisture. Such levels of variation would have practical relevance as raised excreta moisture can increase the relative humidity and ammonia concentrations in controlled environment housing, provide a more favourable environment for fly larvae development, increase the survival of viruses, increase storage and disposal costs of poultry manure and increase the proportion of dirty eggs produced by a laying flock.

A further experiment demonstrated that raised excreta moisture caused by diet gave linear increases in both the number of dirty eggs (Table 10.3.) and the microbial contamination of the shells of eggs (Table 10.3.). Under EC marketing regulations class A eggs should be clean and

Table 10.1. Summary of experimentally derived estimates describing the relationship between dietary composition and excreta moisture in laying hens

Dietary component	Chapter	Reggression coefficiant	SEM[1]	r^2	Significance[2]
Sodium	5	Y=719.65+8.12±1.560X	71.500[a]	0.89	***
Potassium	5	Y=570.76+11.95±2.016X	33.230[a]	0.90	***
Available phosphorus	5	Y=723.50+5.59±0.308X	23.100[a]	0.99	***
Calcium	5	Y=755.47+(-1.24±1.150)X	42.900[a]	0.20	NS
Crude protein	6	Y=410.86+1.32±0.076X	35.360[a]	0.92	***
Lysine	6	Y=597.10+0.526±0.073X	41.000[b]	0.93	***
Methionine	6	Y=651.85+(-0.088±0.125)X	37.590[c]	0.20	NS
Lysine availability	6	Y=595.93+0.154±0.048X	24.300[a]	0.72	***
Resistant starch	7	Y=679.97+0.170±0.029X	26.470[a]	0.90	***
Neutral detergent fibre	7	Y=683.60+0.898X	43.450[d]	1.00	***
Fat	8	Y=692.54+(-0.499±0.142)X	32.520[c]	0.86	***

1. Error df = a) 39 b) 81 c) 45 d) 94
2. Significance level of the slope (b) where y=a+bx *** ($p<0.001$), ** ($p<0.01$), * ($p<0.05$), NS ($p>0.05$)

Table 10.2. Summary of experimentally derived estimates describing the relationship between dietary composition and water intake in laying hens

Dietary component (g/kg)	Chapter	Reggression coefficient	SEM[1]	r^2	Significance[2]
Sodium	5	Y=130.74+13.67±1.320X	94.110[a]	0.96	***
Potassium	5	Y=174.16+9.19±0.604X	69.600[a]	0.98	***
Available phosphorus	5	Y=192.07+7.43±6.384X	23.100[a]	0.97	***
Calcium	5	Y=204.19+(-0.41±0.801)X	53.790[a]	0.26	NS
Crude protein	6	Y=188.90+0.746±0.158X	50.130[a]	0.92	***
Lysine	6	Y=153.23+0.405±0.109X[3] Y=265.62+0.199±0.220X[3]	36.310[b]	0.77 0.17	***
Methionine	6	Y=322.93+0.684±1.400X	31.160[c]	0.68	***
Lysine availability	6	Y=44.68+0.233±0.034X	25.450[a]	0.92	***
Resistant starch	7	Y=280.21+0.177±0.042X	37.240[a]	0.82	***
Neutral detergent fibre	7	Y=101.13+1.16X	43.560[d]	1.00[4]	*
Fat	8	Y=202.49+0.164±0.105X	26.850[e]	0.55	NS

1. Error df = a) 39 b) 81 c) 45 d) 94
2. Significance level of the slope (b) where y=a+bx *** ($p<0.001$), ** ($p<0.01$), * ($p<0.05$), NS ($p>0.05$)
3. Estimate of the effect of lysine concentration on water intake at 160 and 220 g/kg crude protein respectively
4. Determined using only two data sets although there was no evidence to suggest the response wouldnot be linear at higher concentrations

Table 10.3. Summary of experimentally derived estimates describing the relationship between excreta moisture, dirty egg output and microbial contamination of first quality eggs from laying hens

Component	Chapter	Reggression coefficiant	SEM[1]	r^2	Significance[2]
Dirty egg output (%)	9	Y=-19.92+0.0367±0.0043X	1.990	0.77	***
Microbial contamination of first quality egg shells $Log_{10}(No/cm^2)$	9	Y=-2.30+0.0081±0.0094X	0.340	0.77	***

1. Error df = 18 (both parameters)
2. Significance level of the slope (b) where y=a+bx *** ($p<0.001$), ** ($p<0.01$), * ($p<0.05$), NS ($p>0.05$)

have not been cleaned (Anon, 1993 b). Dirty eggs are therefore on average marketed for approximately one third of the price of first quality eggs. Increased excreta moisture through variation in diet could therefore result in economic loss to the producer through increased downgrading of eggs and increased microbial contamination of all the eggs. A 100 g/kg increase in the moisture content of excreta for example would result in a 2.5% reduction in egg income from a flock. This experiment therefore demonstrated that there are benefits to the producer in controlling excreta moisture.

The nutrients that were demonstrated to significantly increase excreta moisture when provided in excess of their requirements were sodium, potassium, phosphorus, crude protein, lysine and resistant starch (Table 10.1.) whereas increases in dietary calcium, methionine and fat had no effect on excreta moisture outputs (Table 10.1.). The quantitative estimates derived for the effects of those nutrients that effected excreta moisture indicated that their responses were without exception linear. Previous workers have failed to standardise environmental and collection procedures during dry matter determination of excreta which makes comparison of literature imprecise. Standardisation of collection method and correction for loss of moisture to the environment as carried out in these experiments has allowed this problem to be overcome. Therefore from a comparison of the quantitative estimates (Table 10.1.) from individual caged laying hens it can be concluded that the order of importance of dietary components examined in determining excreta moisture when expressed per unit weight were potassium > sodium > inorganic (available) phosphorus > crude protein > lysine > resistant starch. Examination of the individual estimates suggest the minerals, particularly sodium and potassium are of most importance. Further experiments examined the effects of excessive heat treatment of protein concentrates on excreta moisture and the effects of other factors such as the anion in sodium salts (chloride or bicarbonate), different cereal sources (wheat barley and maize), levels of wheat bran, fat source and level of oxidation on excreta moisture. Heat treatment of a protein concentrate

reduced excreta moisture output and increased concentrations of wheat bran increased excreta moisture, however the other factors had no effect.

In practice the order of ranking may differ from that when expressed per unit weight depending on the amount of variation each is subject to in the diet. Sodium concentration of plant material is low and therefore dietary sodium is generally provided in diets as the free chloride salt. As a comparatively low priced and non - toxic additive it is subject to variation due to formulation mistakes as well as separation from the bulk of the diet during storage. Potassium is generally present in proprietary rations far in excess of the nutrient requirement of 1.5 g/kg (National Research Council, 1984) and is subject to considerable variation as potassium content of plant material is high. Crude protein levels of the diet are also subject to considerable variation particularly when protein sources of low biological value are incorporated when protein prices are high. When this is taken into account the nutrients of greatest practical importance in causing high excreta moisture levels were potassium, sodium, crude protein, phosphorus, lysine and resistant starch. In practical feed formulation there needs to be a more precise control of variation in dietary sodium and potassium levels. There also needs to be a reduction in the concentration of components that are rich in crude protein. This may involve a more widespread use of synthetic amino acids to lower crude protein concentrations in ration formulations whilst still meeting the animals requirement and a reduction in the use of proteins of low biological value when protein prices are high.

Not all possible interactions between nutrients were examined. However there were no methionine x lysine or lysine x crude protein interactions or sodium x phosphorus interactions in the practical range. However in diets containing high concentrations of sodium and phosphorus there was a treatment interaction which was consistent with observations of Kando and Ross (1962 b) and Hijikuro (1976) who both observed a limitation on water excretion at high levels. The latter

authors have also demonstrated an additive effect of sodium and potassium on excreta moisture.

Experiments in this project have therefore shown that variation in dietary nutrient composition is a major factor effecting excreta moisture and that variation in excreta moisture with diet can effect the number of dirty eggs produced by caged laying hens. If it is assumed that all the nutrients examined act additively and that feed intakes are constant then a linear model can be developed using the linear estimates obtained in experimental work to predict the variation in the proportion of dirty eggs produced in laying houses from the variation in nutrient composition of diets fed (Figure 10.1.).

Effect of diet on dirty egg output

To test the ability of the model to predict variation in dirty eggs in laying hen flocks and confirm that in practice variation in dirty eggs are due to diet a study examined the incidence of dirty eggs in commercial egg production units fed the same ingredient formulation manufactured at different feed mills. Feed composition and egg quality data were obtained from 24 commercial laying flocks of 40000 birds of 35-45 weeks of age housed in Potter or Dutchman cages under a 14L: 10D lighting regime and subsequently analyzed according to Appendix 14. The experimentally determined nutrient analysis of the 24 feeds (Appendix 15) were fitted to equation 10.1. (Figure 10.1.) to predict the level of excreta moisture for each site (Appendix 16). The predicted excreta moistures were fitted to equation 10.2. (Figure 10.1.) to predict the proportion of dirty eggs for each site (Appendix 16). Data were fitted to a simple linear regression model with predicted dirty egg numbers as independent variables and experimentally determined dirty egg numbers as dependent variables to test for a relationship between predicted and experimentally determined dirty eggs. There was a linear ($p<0.025$) relationship ($Y=-1.056+0.497\pm0.207X$) between predicted and experimentally determined dirty egg numbers (Figure 10.2.). Variation in predicted

Figure 10.1. A model to predict the proportion of dirty eggs from diet

Equation 10.1. Prediction of excreta moisture from diet

$$Y = 697.70^{10} + (8.13 \times X1^{.216}) + (11.95 \times X2^{.248}) + (5.59 \times X3^{.238}) + (1.32 \times X4^{.238}) + (0.511 \times X5^{.15}) + (0.154 \times X6^{.15}) + (0.170 \times X7^{.143}) + (0.898 \times X8^{.2268})$$

Equation 10.2. Prediction of the proportion of dirty eggs from excreta moisture

$$Y = -19.92 + 0.0367 \times X9^{7}$$

1. The constant represents the level of excreta moisture of a typical wheat soya based laying hen diet (Table 5.1.)

2. Independent variables X1-X4 and X8 are calculated as g nutrient/kg diet at requirement - g nutrient/kg diet determined experimentally, and represent

X1 Sodium

X2 Potassium

X3 Phosphorus

X4 Crude protein

X8 Neutral detergent fibre

3. Nutrient requirements suggested by the National Research Council (1984) and the Agricultural Research Council (1975) (Appendix 1.) are used in determination of the independent variables

4. The nutrient requirement for potassium (1.5 g/kg) is lower than can be achieved using practical ingredients. Trial formulations suggest concentrations of potassium below 4.5 g/kg cannot be achieved and therefore this more realistic minimum value is used in the model

5. Independent variables X5 - X7 are calculated by either g/kg crude protein, g available lysine/kg lysine or g resistant starch/kg total starch and represent

X5 Lysine

X6 Amino acid availability

X7 Resistant starch

6. Neither the National Research Council (1984) or the Agricultural Research Council (1975) provide recommended dietary concentrations for Resistant starch or Neutral detergent fibre. Trial formulations of maize based diets indicate minimum values of 1.10 g/kg total starch for resistant starch and 90 g/kg for neutral detergent fibre

7. X9 is the estimate of excreta moisture from equation 10.1.

8. Nutrient requirements or minimum values used in the model are indicated in Appendix 13

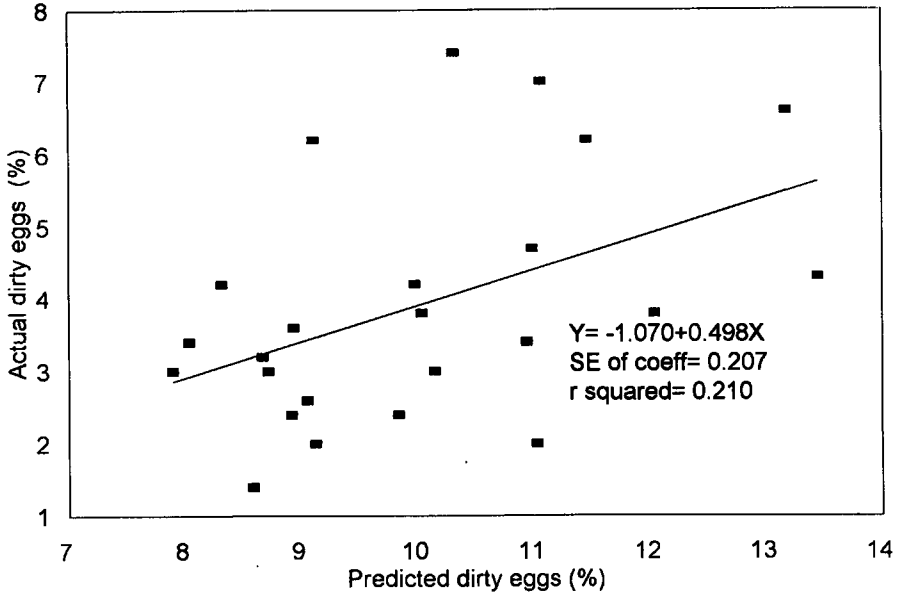


Figure 10.2. A comparison of predicted and experimentally determined dirty egg numbers of commercial laying flocks

proportions of dirty eggs accounted for 18.60% ($r=0.456$) of the variation in experimentally determined dirty egg numbers (Appendix 17). Variation in diet, within the restrictions imposed could therefore account for a significant proportion of variation in dirty egg numbers between laying houses. Diet is therefore a significant factor in accounting for the variation in dirty egg numbers between laying houses.

To determine the factors which were effecting the dirty egg numbers in this experiment the regression analysis was repeated using variation in dirty eggs due to individual nutrient variation as independent variables. Linear relationships between both sodium and neutral detergent fibre concentration and experimentally determined dirty egg numbers were shown which accounted for the majority of variation due to diet. Multiple regression analysis showed that no further variation could be accounted for by including both as independent variables in the regression model as they were correlated ($r=0.68$) ($p<0.01$). Variation in dietary potassium, available phosphorus and crude protein concentration had no effect ($p>0.05$) on the proportion of dirty eggs in the experiment as the amount of variation for these nutrients was small. Therefore in this experiment variation in dietary sodium was the main factor effecting excreta moisture although in other situations variation in another factor could be important.

Few feed manufacturers analyse each batch of ingredients for use in formulation and therefore differences in actual and predicted dietary mineral concentrations in feeds are known to be large in both raw materials and finished feed as are levels of crude protein in protein concentrates (Austic and Patience, 1988). In order to control a significant proportion of the level of variation in dirty egg numbers between laying hen flocks, producers and feed manufacturers must therefore attempt to control excessive levels of dietary nutrients, with particular emphasis being placed on potassium, sodium and available phosphorus content of the diet and the minimisation of excess crude protein levels through increased use of synthetic amino acids in the diets of laying hens.

In conclusion this model has shown that variation in diet from expected requirements is a major factor causing variation in excreta moisture and dirty egg output of caged laying hens. There are therefore economic advantages to the producer for controlling variation in dietary composition from expected requirement.

CHAPTER 11.

GENERAL CONCLUSIONS

(i) Variation in dietary nutrients in excess of expected requirements gave a large variation (550 -900 g/kg) in excreta moisture of laying hens. Such variation is practically important as increased excreta moisture can increase relative humidity and ammonia concentrations in controlled environment housing, provide a more favourable environment for fly larvae development and may cause economic loss in egg production systems through an increase in the number of dirty eggs. High relative humidity can lead to rapid deterioration of house structure and electrical equipment and increased survival of viruses which cause respiratory disease. The increased volume of high moisture excreta produced could also increase the storage and disposal costs of poultry manure from an egg production unit.

(ii) An experiment has shown that increases in excreta moisture caused by diet can give linear increases in both the number of dirty eggs produced and the microbial contamination of first quality egg shells. A high proportion of dirty eggs reduces the economic efficiency of a flock of laying hens. Dirty eggs are on average marketed for approximately one third of the price of first quality eggs. A 100 g/kg increase in the moisture content of excreta would result in a 2.5% reduction in egg income from a flock.

(iii) Experiments have been able to quantitatively describe the effects of increasing amounts of dietary nutrients when provided in excess of the assumed requirements of laying hens on excreta moisture.

(iv) Nutrients that were demonstrated to significantly increase excreta moisture when provided in excess of their requirements were:

- Sodium
- Potassium
- Available Phosphorus
- Resistant starch
- Crude protein
- Lysine

Increases in the concentrations of dietary calcium, methionine and fat however, had no effect on excreta moisture outputs.

(v) Excessive heat treatment of a protein concentrate decreased excreta moisture output whereas other dietary factors such as the anion (bicarbonate or chloride) in sodium salts, different cereal sources and the degree of fat oxidation and saturation had no effect on excreta moisture.

(vi) The quantitative estimates of the effects of each nutrient that affected excreta moisture indicated that their responses were without exception linear.

(vii) The order of ranking of those nutrients that effected excreta moisture when increased per unit weight was potassium > sodium >phosphorus > crude protein > lysine > resistant starch.

(viii) The factors of greatest practical importance in causing high excreta moisture levels were dietary potassium, sodium and crude protein.

(ix) A study that examined the incidence of dirty eggs in commercial egg production units fed diets with the same ingredient formulation but manufactured at different feed mills showed that there was a significant relationship between the variation in the levels of dirty eggs observed between units and the variation predicted using a model that predicted dirty egg numbers from dietary nutrient analysis. The relationship was explained by variation in excess levels of dietary sodium.

(x) The study has therefore shown that excreta moisture can be markedly reduced by diet and that attention to minimising excess dietary levels of potassium, sodium and crude protein in practical laying hen diets would significantly reduce the incidence of excreta contaminated eggs in the UK egg industry.

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APPENDICES

APPENDIX 1

Both the Agricultural Research Council (1975) and National Research Council (1984) have produced tables of recommended requirements for the laying hen. Both state that these do not include margins of safety to allow for losses during manufacture or storage. The table below compares the recommended requirements of the two authorities.

Table A Comparison of Agricultural Research Council (1975) and National Research Council (1984) guidelines for the nutrient requirements of the laying hen

	Nutrient requirement (g/kg) of laying hen			
	ARC		NRC	
Component				
Crude protein	165.00		150.00	
Calcium	35.00		32.50	
Phosphorus (available)	3.50		5.00	
Phosphorus (total)	.....		5.00	
Sodium	1.00		1.50	
Potassium	2.50		1.50	
Chloride	0.90		0.80	
Magnesium	0.40		0.60	
		g/kg crude protein		g/kg crude protein
Lysine	7.50	45.45	6.00	40.00
Methionine	3.50	21.21	2.70	18.00
Methionine+cys	4.70	28.48	5.00	33.33
Tryptophan	1.70	10.30	1.10	7.33
Glycine+serine	.....	.....	5.00	33.33
Leucine	6.80	41.21	12.00	80.00
Isoleucine	5.50	33.33	5.00	33.33
Valine	5.50	33.33	5.00	33.33
Histidene	1.70	10.30	2.20	14.66
Arginine	5.10	30.90	8.00	53.33
Phenylalanine	3.90	23.63	4.00	26.66

APPENDIX 2

The determination of sodium concentration in blood serum (Colorimetric Method)

The chromolyte colorimetric method of Miles Inc. (Diagnostics division Tarry Town, NY 10591-5097 USA) was used with a Technicon RA- 1000 blood analyser at 37 °C. Quality control of the analyser was carried out using the Technicon test point assayed chemistry controls 1 and 2 (product T03-1220-62 and T03-1221-62 respectivly).

Principle The assay involves use of a sodium selective macrocyclic compound known as chromogenic cryptahemispherand [1.1]. The chromogenic cryptahemispherand [1.1] compound is composed of a highly selective sodium ionophore covalantly linked to an ionisable chromophoric group. At constant pH maintained by a buffered reagent the chromophoric group exhists in an equilibrium of its protonated and unprotonated forms, which have distinctly different absorption spectra. Complexation of sodium ion by the chromogenic ionophore induces a change in the basicity of the chromophoric group and hence a change in the ratio of its two spectrally different forms. A color change is observed at 500 nm, the rate of change in absorbance being proportional to the sodium concentration in the sample. Calibration was carried out according to the Technicon method No SM4-0187E94 using a Technicon SET point calibrator, product number T03-1291-D0.

The method was linear from 80 mmol/L to 180 mmol/L.

APPENDIX 3

The determination of uric acid concentration in blood serum

Determination of uric acid in serum was carried out using a Technicon RA 1000 blood analyser according to method number SM4-0187E94 (Miles inc., Diagnostics division Tarry Town, NY 10591-5097 USA). Quality control of the analyser was carried out using the Technicon test point assayed chemistry controls 1 and 2 (product T03-1220-62 and T03-1221-62 respectivly).

Principle The enzyme uricase (EC 1.7.3.3) catalyses the oxidation of uric acid to allantoin thereby generating hydrogen peroxide. In a reaction with 4-aminoantipyrine and a phenol derivative, a red colur is produced which is directly proportional to the uric acid concentration in the sample. This is monitered at 500 nm.

$$2\,H_2O + \text{Uric Acid} + O_2 \xrightarrow{\text{Uricase}} H_2O_2 + CO_2 + \text{Allantoin}$$

$$H_2O_2 + \text{4-aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Chromophore} + H_2O$$

Calibration was carried out according to the Technicon method No SM4-0187E94 using a Technicon SET point calibrator, product number T03-1291-D0. The method is linear from 2 mg/dL (119 umol/L) to 25 mg/dL (1487umol/L).

APPENDIX 4

Determination of uric acid in avian excreta by use of uricase and differential spectrophotometry.

The method used was based on that described by Pudelkiewicz *et al* (1969) and involved a simple extraction and dilution followed by absorbance measurements before and after reaction with uricase.

Uric acid standards Prepared by dissolving 100 mg of uric acid in 12 ml of 0.50% lithium carbonate in a 100 ml volumetric flask at 60 °C to produce a stock solution. The sample was cooled and made up to volume with water. Serial dilutions of the stock solution were made with glycine buffer to give concentrations of uric acid between 1 and 10 ug per ml. A standard curve was prepared by plotting absorbance at 292 mu against concentration of uric acid.

Procedure One gram samples of excreta, ground to pass a 0.5 mm mesh screen were quantitatively transferred to 250 ml volumetric flasks, to which were added two 25 ml portions of 0.50 % lithium carbonate solution. Samples were extracted for 30 minutes, and made up to volume with distilled water. A portion was subsequently centrifuged at low speed, and a 1:10 dilution of an aliquot of each sample was made with 0.1 M, pH 9.2 ± 0.1 glycine buffer. One millilitre aliquots of each of the diluted uric acid samples were pipetted into 15 ml tubes. A blank was prepared using 1 ml of the glycine buffer and 9 ml of uricase solution (40 mg uricase to 1 litre of 0.1 M glycine buffer). Absorbance measurements were taken for the blank and for samples following addition of 9 ml of uricase solution at 292 mu. After all the samples were read they were capped and incubated at 45 °C for 4 hours. After incubation, terminal absorbance

measurements were taken and cell corrections made, using the incubated blank.

Calculations Concentration (mg uric acid/ gm excreta) of uric acid in excreta were calculated as follows.

$$\frac{E\text{ (initial)} - E\text{ (terminal)} \times \text{dilution factor}}{K \times \text{sample weight} \times 1000}$$

APPENDIX 5

Effect of dietary sodium concentration on the serum sodium concentration of the laying hen

This appendix describes data obtained as a secondary objective of experiment 13 (chapter 9). The objective was to quantitatively examine the effect of increased dietary sodium concentration on serum sodium concentration, as a means of understanding the mechanism of dietary sodium concentration on excreta moisture of laying hens.

Bird management, diets used, and experimental layout were identical to those previously described for experiment 13. Blood samples were removed by puncture of the wing vein of 288 laying birds (12 per plot) approximately six - seven hours after the start of the light period, on day 72 of the experiment to avoid variation in blood osmolarity with egg laying (Howard, 1975). Samples were allowed to clot, were centrifuged at 1500 g for 15 minutes and had serum removed. Samples were frozen at -20± 1° C until assayed. Serum was analysed for sodium concentration using a Technicon RA-1000 blood analyser according to the method of miles inc (Appendix 2).

Results (see Table)

Increased dietary sodium concentration had no effect ($p>0.05$) on feed intakes and therefore gave linear increases ($p<0.001$) in daily sodium intake. There were however no effects ($p>0.05$) of increased dietary sodium concentrations on serum sodium concentration.

Increased dietary sodium concentration had no effect on serum sodium concentration, consistent with observations of Gilman (1937) in dogs when allowed to drink freely. Sodium accounts for 90% of extracellular fluid osmolarity (Fitszimmons, 1969). Increased dietary sodium concentrations will increase extracellular fluid osmolarity, stimulating water intake and excretion

of excess sodium. In conclusion the osmotic work of the kidneys, and increased intake of water, are more than sufficient to dilute serum sodium ion concentration to its original concentration in the laying hen.

Table Effect of dietary sodium concentration on serum sodium concentration and other parameters of laying hens

	Dietary sodium (g/kg)					
Parameter	1.60	5.00	10.00	15.00	SEM	Significance[3]
Sodium intake (g/kg)	1.75	5.54	11.01	16.14	0.930[1]	***
Serum sodium (mEq/l)	167.80	186.10	178.50	186.10	13.1801[2]	NS
Excreta moisture (g/kg)	732.70	768.00	836.10	856.50	23.13[1]	***

1. Error df=18
2. Error df=144
3. Significance level of the slope b where y=a+bx *** ($p<0.001$), NS ($p>0.05$)

APPENDIX 6

Nutrient composition of dried whole egg protein; a comparison with laying hen requirements

The essential amino acid composition of the mixed proteins of dried whole egg are presented in the enclosed table. It is apparent from examination of the table, that eggs are relatively high in many of the essential amino acids. These amino acids are also present in approximately the right proportions for optimum production of the laying hen, and consequently this protein source has a high biological value.

Nutrient	g/kg[2]	g/kg CP[2]	Requirement (g/kg CP)[1]
MJ of ME/kg	25.28		
Crude protein	474.00		
Crude fat	431.00		
Ash	4.00		
Argenine	30.40	64.13	30.90
Methionine + cystine	26.50	55.91	28.48
Glycine + serine	15.80	33.33	33.33
Histidine	11.50	24.26	10.30
Isoleucine	26.10	55.06	33.33
Leucine	41.10	86.71	41.21
Lysine	32.40	68.35	45.45
Methionine	15.40	32.49	21.21
Phenylalanine	25.30	53.37	23.73
Threonine	23.30	49.15	26.20
Tryptophan	7.50	15.82	10.30
Valine	31.20	65.82	33.33
Calcium	2.09		
Sodium	5.10		
Potassium	5.33		
Phosphorus	7.98		
Magnesium	0.45		
Chloride	6.91		

1. Requirements of laying hen according to Agricultural Research Council (1975)
2. Courtesy of Framptons Ltd, Shepton Mallett, Somerset, UK

APPENDIX 7

The in vitro starch digestibility of some carbohydrate containing foods

The following table indicates the *invitro* starch digestibility of some carbohydrate foods *(adapted from Englyst et al, 1992)*

	Types of starch (g/100g of DM)			
	RDS[1]	SDS[1]	RS[1]	TS[1]
Sample				
White bread	69	7	1	77
Corn flakes	73	2	3	78
Boiled potato (cold)	53	11	10	75
Potato starch (raw)	6	19	75	99
Banana flour	3	15	57	75
White wheat flour	40	39	2	81
Pearl barley (boiled/cold)	34	30	9	73
Millet (boiled/cold)	42	28	6	75
Peas (frozen/boiled)	12	2	5	20
Lentils (boiled/cold)	24	22	9	54
Haricot beans (boiled)	8	19	18	45
Bean flakes	27	16	6	49
Soluble starch	100	0	0	100
Glucose	100	0	0	0

1. RDS, rapidly digestable starch, SDS, slowly digestable starch; RS, resistant starch; TS, total starc

APPENDIX 8

A classification of starch for nutritional purposes

The Englyst resistant starch kit (Novo Biolabs) was used for the quantification of the various starch fractions, using the method of Englyst and Kingman, based on the classification of starch for nutritional purposes of Englyst and Cummings (1987 b).

Principle The starch fractions were quantified by controlled enzymic hydrolysis, and measurement of the released glucose, using glucose oxidase.

Total starch (TS) Following gelatinisation in boiling water, and treatment with KOH to disperse retrograded starch, TS was measured as the glucose released by complete enzymic hydrolysis of starch.

Rapidly digestable starch (RDS) and slowly digestable starch (SDS) These fractions were measured after incubation with pancreatic amylase and amyloglucosidase at 37 °C. A value for RDS was obtained as the glucose released after 20 minutes and SDS as the glucose released after a further 100 minutes incubation.

Resistant starch (RS) This was calculated as the starch not hydrolysed after 120 minutes

Measurement of the released glucose was carried out using a glucose oxidase (GOD-PAP) diagnostic kit (Merckotest glucose (GOD-PAP). BDH, Cat. No. 25456 4P). B-D-glucose: oxygen-1-oxidoreductase, EC 1.1.3.4 (GOD) catalysed the oxidation of glucose in accordance with the following equation

GOD

Glucose + O_2 + H_2O <----------> gluconic acid + H_2O_2

The hydrogen peroxide formed in the reaction reacted with aminoantipyrine and 4-hydroxybenzoic acid in the presence of hydrogen peroxide- oxidoreductase, EC 1.11.1.7 to form N-(4-antipyryl)-P-benzoquinone imine. The addition of mutarotase accelerates the reaction. The amount of dye formed is proportional to the glucose concentration.

APPENDIX 9

Fatty acid proportions (mmol/ mol) of beef tallow and soya bean oil (Larbier and Leclercq, 1994)

		Beef tallow	Soya bean oil
Saturated	4:0	0	0
	6:0	0	0
	8:0	0	0
	10:0	0	0
	12:0	0	0
	14:0	70	0
	16:0	290	95
	18:0	210	37
Unsaturated	18:1	410	217
	18:2	20	571
	18:3	0	65

APPENDIX 10

Between experiments and during acclimatization to experimental housing birds were fed the following diet

Table Composition (g/kg) and analysis of basal commercial laying hen ration

Component	g/kg
Barley	65.0
Wheat	337.0
Low oil maize germ	240.0
Prairie meal	15.0
Soya extract hi-protein meal	135.0
Full fat soya (extruded)	87.5
D-L methionine	0.6
Limestone	100.0
Vitamin mineral premix	20.0
Analysis (calculated)	
Oil	39.2
Protein	170.1
Fibre	32.8
Ash	140.7
Total lysine	8.6
Available lysine	7.9
Methionine	4.4
Methionine + cystine	7.3
Tryptophan	2.1
Calcium	42.9
Phosphorus	5.9
Available phosphorus	3.6
Sodium	1.7

APPENDIX 11

The modified true metabolisable energy (TME) procedure of Mc Nab and Blair (1988)

In all experiments, adult medium body weight, brown egg laying strain cockerels aged between 25 and 52 weeks were used. The cockerels were kept in individual cages with individual eating and watering facilities in a controlled environment (20 °C). When not under experiment the birds had access *ad libatum* to a low residue maintenance diet.

On day one of each assay, 24 cockerels had their food withdrawn and were allocated to treatments. After 24 h each bird was given 50 ml of a 70 % sucrose solution by tube, to partially alleviate the effects of starvation. After a further 24 h each bird was fed 50 g of the appropriate test feed or sucrose (control for determination of endogenous losses). Clean trays were subsequently inserted beneath the cages. After a further 24 h each bird recieved 50 ml of water by tube, to overcome any effects induced by low water intake. After 48 h all excreta voided were collected quantitatively, weighed and dried at 60 °C to determine the dry matter content. Excreta and samples of feeds were ground to pass a 0.5 mm mesh screen and subsequently analysed by the methods described in the text.

APPENDIX 12

Virus isolation and clinical signs on one farm in Northern Ireland

Age (days)	Direct electron microscopy of faeces	Cell culture	Litter and clinical condition
1	2/6 ELP[a]	None	Excellent
6	7/8 ELP[a]	2/6 Reovirus 2/6 Adenovirus	Litter very wet
13	6/8 Rotavirus	7/7 Reovirus	Litter drying
20	5/7 Rotavirus	7/7 Reovirus	Litter very wet
27	2/6 ELP[a] 1/6 Adenovirus	2/5 Reovirus 2/5 Adenovirus	Litter drying
34	2/6 ELP[a]	1/6 Reovirus 5/6 Adenovirus	Litter very wet
41	NVO[b]	4/4 Adenovirus	.....
48	1/3 Adenovirus	3/4 Reovirus 1/4 Adenovirus	Litter drying
56	NVO[b]	.....	Litter good

An adaption of work by Mc Ferran *et al* (1983) cited in Pattison (1989)

a. Enterovirus like particles. Numerator = number of specimens possitive; denominator = number examined

b. No virus isolated

APPENDIX 13

Nutrient composition (g/kg) of the basal diet used in the prediction model (equation 10.1.)

Component	g/kg
Sodium	1.50
Potassium	4.50
Phosphorus	4.00
Crude protein	165.00
Neutral detergent fibre	90.00
Resistant starch[1]	1.10

1. Resistant starch was expressed as g/kg total starch

APPENDIX 14

Validation of a model to predict dirty egg numbers by diet

Introduction The objective of this experiment was to test the ability of a quantitative model (Figure 10.1.) to predict variation in dirty eggs in laying hen flocks from diet composition by using commercial data from 24 flocks of laying hens.

Materials and Methods

Bird management Feed composition and egg quality data were obtained from twenty four commercial laying hen flocks of 40000± 1000 ISA brown laying hens aged between 35 and 45 weeks of age and housed in groups of five in either Potter or Dutchman cages under 14L :10D lighting regimes. Birds were fed diets formulated to meet the nutrient requirements of the laying hen and had *adlibitum* access to the diet and 2 nipple drinkers throughout the duration of the experiment.

Egg and feed measurements Samples of feed were removed from the storage bin of each of the twenty four laying houses. Simultaneously 200 eggs were removed at random from each of the top right, middle right, top left, middle left and bottom tier collection cradles of the laying cages for each house. The proportion of dirty eggs were recorded for each tier using Ministry of Agriculture Fisheries and Foods guidelines and used to calculate an estimate of the number of dirty eggs produced for the whole house.

Feed analysis Samples of feeds from each house were ground to pass a 0.5 mm mesh screen and

were subsequently analyzed by standard proximate analysis techniques for dry matter, ash, ether extract and crude protein (A.O.A.C, 1990) and for total water insoluble fibre by neutral detergent fibre determination according to the method of Van Soest and Wine (1967) using fibertec equipment. Sodium, calcium, phosphorus and potassium concentrations were determined with an atomic absorption spectrophotometer (Smith Hiefte 1000, Thermo Jarrell Ash Corporation) following wet digestion (A.O.A.C, 1990). Inorganic phosphorus was determined using a colorimetric technic (MAFF, 1986) in which concentration of phosphorus in a trichloroacetic acid extract is determined spectrophotometrically as the yellow phospho- vanado- molybdate complex at 400 nM. Total starch, resistant starch, slowly digestible starch and readily digestible starch fractions were quantified with an Englyst resistant starch kit (Novo biolabs) (Appendix 8).

Although quantitative estimates for dietary lysine concentration and amino acid availability were determined and included in the prediction equation 10.1. they were not included in the current validation of the model. The estimate of the slope for amino acid availability was small, ambiguous and the expense of carrying out analysis of amino acid availability on a routine basis expensive. In addition in the commercial situation crude protein with high amino acid availability is favoured for maximising profit margins, whereas lowered excreta moisture would favour crude protein with lowered amino acid availability. The estimate of the effect of dietary lysine concentration on excreta moisture was also not included due to the small estimate and high cost of analysis.

Statistical analysis The experimentally determined nutrient analysis of the twenty four feeds (Appendix 14) were fitted to equation 10.1. to predict excreta moisture for each site (Appendix 15.). The predicted excreta moistures were fitted to equation 10.2. to predict the proportion of dirty eggs for each site (Appendix 15.).

To test the ability of the model to predict variation in dirty egg numbers in commercial laying houses, data were fitted to a simple linear regression model with predicted dirty egg numbers as independent variables and experimentally determined dirty egg numbers as dependant variables to test for a relationship between predicted and experimentally determined numbers of dirty eggs.

APPENDIX 15

A summary of experimentally determined analysis (g/kg) of commercial feeds and determined dirty egg numbers for prediction model

Table 1. Experimentally determined analysis (g/kg) of commercial feeds and determined dirty egg numbers for prediction model (diets 1-12)

Site	Tarry[3] eggs(%)	Hen soiled eggs(%)	Total dirty eggs(%)	Dry matter	Ash	Crude protein	Ether extract	Sodium	Potassium	Phosphorus	TS[1]	RS[2]	SDS[2]	RDS[2]	Neutral detergent fibre
1	2.80	3.80	6.60	871.50	120.90	186.40	79.70	4.80	10.10	3.59	298.79	11.80	570.80	417.38	148.71
2	3.20	3.80	7.00	898.50	169.00	178.30	49.50	4.30	7.70	3.83	302.80	12.50	603.70	383.82	134.31
3	0.40	3.40	3.80	895.20	114.50	183.50	59.00	5.10	8.50	3.59	322.10	13.60	626.45	359.95	142.48
4	0.60	2.80	3.40	914.10	142.20	177.50	41.50	4.50	9.00	3.70	236.29	0.00	477.62	522.37	123.07
5	1.00	1.40	2.40	888.80	115.80	177.30	77.40	2.20	8.50	3.94	301.23	1.11	530.92	467.96	144.95
6	0.20	1.20	1.40	882.70	122.90	177.60	54.30	1.50	9.40	3.43	257.13	0.00	447.67	552.33	124.48
7	0.80	1.60	2.40	887.70	114.50	163.30	69.20	3.70	8.70	4.55	286.57	14.91	668.61	316.47	134.96
8	0.60	1.40	2.00	864.90	141.40	180.40	78.20	3.50	7.30	3.45	293.67	0.00	502.29	497.70	117.22
9	1.70	3.00	4.70	886.60	131.50	161.00	47.30	3.30	8.60	3.39	281.97	0.00	327.48	672.52	153.47
10	1.70	4.50	6.20	883.90	102.90	164.30	55.30	4.10	9.40	4.42	354.24	1.19	651.42	322.76	147.00
11	1.00	3.20	4.20	856.50	142.90	169.80	61.80	3.00	7.40	4.27	256.74	0.00	373.68	626.31	148.61
12	1.30	3.00	4.30	861.20	123.20	181.00	68.10	4.60	13.30	4.23	311.36	12.20	489.17	498.62	140.81

1. TS= total starch
2. RS= resistant starch, SDS= slowly digestible starch, RDS= rapidly digestible starch expressed as g/kg of total starch
3. Tarry eggs- eggs contaminated with enteric excreta only

Table 2. Experimentally determined analysis (g/kg) of commercial feeds and determined dirty egg numbers for prediction model (diets 12 - 24)

Site	Tarry[3] eggs (%)	Hen soiled eggs (%)	Total dirty eggs (%)	Dry Matter	Ash	Crude Protein	Ether extract	Sodium	Potassium	Phosphorus	TS[1]	RS[2]	SDS[2]	RDS[2]	Neutral detergent fibre
13	0.60	6.80	7.40	911.50	148.75	186.96	64.10	2.39	10.40	3.52	307.69	3.70	612.14	384.15	115.19
14	0.40	5.80	6.20	908.20	125.75	172.29	85.30	2.30	8.80	3.59	310.97	8.20	624.27	367.52	107.70
15	0.00	3.60	3.60	908.70	163.95	171.15	76.30	2.40	8.10	3.57	297.00	9.60	641.52	348.85	115.66
16	0.60	3.60	4.20	923.60	239.20	155.50	61.90	2.55	9.61	3.10	349.77	6.40	612.94	362.66	113.22
17	0.60	1.40	2.00	902.90	123.00	175.14	71.20	1.70	10.81	3.47	300.52	0.00	539.06	460.90	130.08
18	1.60	1.60	3.20	901.80	145.70	177.80	89.10	2.10	8.77	4.17	384.98	19.20	652.00	328.79	106.78
19	0.80	1.80	2.60	899.70	137.75	180.90	62.30	2.44	9.42	4.32	372.83	21.60	662.21	316.80	106.03
20	1.40	1.60	3.00	905.30	147.80	180.39	71.60	2.00	8.40	4.16	246.33	8.10	557.88	434.01	92.56
21	0.40	3.40	3.80	893.30	127.00	177.65	84.30	2.40	9.87	2.47	346.61	8.40	547.62	443.97	116.57
22	0.60	2.40	3.00	893.70	140.60	182.80	70.70	2.60	9.56	4.15	354.17	7.65	630.80	361.55	101.21
23	1.20	1.80	3.00	891.70	126.15	179.50	93.00	2.05	10.00	3.72	397.53	16.80	638.44	344.75	93.35
24	1.40	2.00	3.40	910.60	164.56	176.20	80.10	2.10	8.90	2.57	337.50	0.00	446.40	553.60	94.40

1. TS = total starch
2. RS = resistant starch, SDS = slowly digestible starch, RDS = readily digestible starch, expressed as g/kg of total starch
3. Tarry eggs - eggs contaminated with enteric excreta only

APPENDIX 16

Predicted excreta moistures and dirty egg numbers, using equations 10.1. and 10.2.

Predicted excreta moistures and dirty egg numbers, using equations 10.1. and 10.2. (diets 1 - 12)

	Nutrient deviation (g/kg)						Model predictions		Experimental values
Site	Sodium	Potassium	Phosphorus	Crude protein	Resistant starch	Neutral detergent fibre	Excreta moisture (g/kg)	Dirty egg numbers (%)	Dirty egg numbers (%)
1	3.30	5.60	-0.41	21.40	10.70	71.31	901.90	13.18	6.60
2	2.80	3.20	-0.17	13.30	11.40	58.10	844.60	11.07	7.00
3	3.60	4.00	-0.41	18.50	12.50	62.01	870.90	12.04	3.80
4	3.00	4.50	-0.30	12.50	-1.10	43.49	840.90	10.94	3.40
5	0.70	4.00	-0.06	12.30	-0.01	37.86	811.00	9.84	2.40
6	0.00	4.90	-0.57	12.60	-1.10	6.53	777.00	8.59	1.40
7	2.20	4.20	0.55	-1.70	1.38	14.49	785.70	8.92	2.46
8	2.00	2.80	-0.55	15.40	-1.10	23.36	791.50	9.13	2.00
9	1.80	4.10	-0.61	-4.00	-1.10	77.37	842.22	10.99	4.70
10	2.60	4.90	0.42	-7.00	0.09	65.79	855.10	11.46	6.20
11	1.50	2.90	0.27	4.80	-1.10	53.92	814.70	9.98	4.20
12	3.10	8.80	0.23	16.00	11.11	49.17	909.30	13.45	4.30

Predicted excreta moistures and dirty egg numbers using equations 10.1. and 10.3. (diets 13 - 24)

	Nutrient deviation (g/kg)						Model prediction		Experimental values
Site	Sodium	Potassium	Phosphorus	Crude protein	Resistant starch	Neutral detergent fibre	Excreta moisture (g/kg)	Dirty egg numbers (%)	Dirty egg numbers (%)
13	0.89	5.90	-0.48	21.96	2.60	18.81	824.00	10.32	7.40
14	0.80	4.30	-0.41	7.29	7.10	23.15	790.90	9.11	6.20
15	0.90	3.60	-0.43	6.15	8.50	27.01	786.50	8.94	3.60
16	1.05	5.11	-0.90	-9.50	5.30	16.29	769.50	8.32	4.20
17	0.20	6.31	-0.53	10.14	-1.10	50.35	843.40	11.03	2.00
18	0.60	4.27	0.17	12.80	16.10	3.96	779.10	8.67	3.20
19	0.94	4.92	0.32	15.90	20.50	-0.60	789.70	9.06	2.60
20	0.50	3.90	0.16	15.39	7.00	-1.09	758.00	7.90	3.00
21	0.90	5.37	-1.53	12.65	7.30	32.50	816.20	10.04	3.80
22	1.10	5.06	0.150	17.80	6.55	23.10	819.40	10.15	3.00
23	0.55	5.50	-0.28	14.50	15.70	-6.71	780.40	8.72	3.00
24	0.60	4.40	-1.43	11.20	-1.10	-0.21	762.00	8.04	3.40

APPENDIX 17

A correlation matrix to summarise the proportion of variation in dirty egg numbers between commercial laying flocks accounted for by variation in individual dietary componants of the prediction model and the complete model.

Actual dirty eggs	1	1.000						
Predicted dirty eggs	2	0.456	1.000					
Sodium	3	0.414	0.754	1.000				
Potassium	4	0.091	0.459	0.046	1.000			
Phosphorus	5	-0.022	0.747	0.240	-0.019	1.000		
Crude protein	6	0.018	0.252	0.067	0.214	0.001	1.000	
Resistant starch	7	0.040	-0.012	0.124	0.159	0.349	0.239	1.000
Neutral detergent fibre	8	0.461	0.838	0.681	0.041	0.033	-0.137	-0.289	1.000
		1	2	3	4	5	6	7	8